FINAL REPORT

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ESTABLISHMENT OF PRODUCTIVE RANGELAND WITH HIGH-YIELDING FODDER SHRUBS

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C) Executive Summary

Overgrazing and poor rangeland management results in declining productivity in semi-arid areas. This program aimed to contribute to rangeland improvement by: 1) introducing and selecting promising fodder species and individual shrubs; 2) making selected shrubs widely available through clonal propagation; and 3) introducing sustainable land-use principles. Field trials of indigenous and introduced shrub species, including adaptation of sustainable land use practices, evaluations of shrubs as fodder sources (growth rates and recovery, palatability and nutritional value) and development of clonal propagation systems were carried out. Trial areas were expanded, using seedlings and clonally propagated selections. The value of combined diets of fodder shrubs and additional energy sources were studied. Protocols developed in the program were applied to root cuttings of plant species and micropropagation of *A. nummularia*, *A. canescens*, *Cassia sturtii*, *Boscia foetida* and *Haloxylon aphyllum*. Difficulties with germination of *Cassia* and *Boscia* seeds were overcome with the application of scarification methods.

A. nummularia, *A. canescens*, and *Cassia sturtii* have potential as fodder crops in revegetation projects. Plant leaves of all shrubs had acceptable dry matter and organic matter digestibilities, as well as fairly low NDF. Differences due to season and location, at least of the *Atriplex* species, demonstrated the importance of these factors when planning fodder budgets. Intake and NDF digestibility of *A. nummularia* increased when barley or maize was used as an energy source supplementation. However, the tendency of the energy sources to increase NDF digestibility diminished when the supplemental level was raised from 15% to 30% and from 30% to 45%. Results suggested that barley and maize supplemented at a level of 15% gave the highest incremental increase in DM and NDF digestibilities. Negative associative effects occurred in the rumen at supplemental levels of 30% and above. *Boscia* leaves had an acceptable level of organic matter digestibility (46%) and contains sufficient concentrations of Ca, Mg and Mn for the requirements of goats but lacks in P, Cu and Zn.

D) Research Objectives

Rangeland-based livestock production is a principal source of income for the inhabitants of semi-arid areas of southern Africa. Overgrazing and poor management of rangeland has led to erosion and desertification. This program aimed to facilitate the establishment of improved fodder reserves in drylands, composed of high-yielding perennial shrubs and trees, which would aid in creating a sound economic base for rural agrarian populations. Specific objectives were to:

1. introduce promising fodder species (shrubs and trees) to target areas in Southern Africa, and conduct performance and palatability trials.
2. introduce and test sustainable land use principles on plots of introduced fodder shrubs.
3. select the most promising plant species on the basis of agronomic traits, palatability and nutritional tests, for cultivation on poor soils in areas where rainfall is irregular.
4. develop procedures for large-scale clonal propagation of the selected shrubs, as the basis for establishing centers in South Africa for fodder shrub propagation and distribution.

E) Methods and Results

E.1. Sites of studies, fodder plants and observations (South Africa)

Plant material used in nutritional value studies included progeny of the "Hatfield Elite" *A. nummularia* plants that had been selected on the basis of sheep palatability. *A. nummularia* is an important fodder shrub for our target regions; however, while rich in protein and minerals, it is limited in energy value. Therefore a study was conducted to evaluate the effects of two energy sources (maize as a slowly fermenting feed and barley as a rapidly fermenting feed in the rumen), as energy supplements in the diet of ruminants. The nutritional value, degradability and fermentation characteristics of the experimental diets were studied. Another study on *A. nummularia* involved determination of morphological and chemical differences between plants that were found in grazing trials to be "most palatable", "less palatable" and "unpalatable" among the F_1 "Hatfield Select" plants.

Cassia seedlings were also produced and planted out in the autumn at Hatfield for nutritive value studies as well as forage characterization. This planting was devastated by a "black frost" in August, necessitating delay in onset of the study until the plants recovered in 2004. Another trial was initiated at Hatfield (Pretoria) to evaluate the growth rate, production and nutritive value of *Sutherlandia*, *Tripteris* and *Cassia* at different stages of development. The proportions of flowers, leaves, young stem and woody stem as well as protein, fiber components and digestibility were evaluated.

<u>Pretoria</u>: Field trials were initiated at Hatfield during the dry season, April – October, to evaluate aspects of water harvesting using catchment basins, the frequency of watering of transplants, the use of protective plastic sleeves for transplants, effects of pruning of transplants, effects of water-absorbent polymers on establishment of plants of *Tripteris*, *Sutherlandia* and *Cassia*. Results indicated differences among the species with respect to drought sensitivity, pruning and unusually cold conditions that occurred in August 2003. The biggest problem that was encountered during these trials was the impact of harvester termites.

<u>Northern Cape</u>: Herbivory from domestic livestock to antelope, hares, rabbits, tortoises, and birds had a strong impact in the Northern Cape. Furthermore, the interpretation of results obtained in the various trials was confounded by an extreme drought in this area, with virtually no effective precipitation during the reporting period. The only exception was at Kammieskroon, in the winter rainfall area of the west coast of South Africa, where rain and snow were recorded in August 2003, following a very dry winter (the usual rainy season. Such events, although episodic, are part of the pattern in such areas and it is becoming increasingly evident that reclamation efforts in such arid zones will only be successful episodically and then only if managers implement strategies to ameliorate the extreme environmental conditions and protect seedlings/transplants from the ever-present herbivory.

<u>Mier, the Kalahari</u>: *Atriplex* species and *Cassia* seedlings were evaluated for production, palatability and nutritional parameters. Evaluations on the adaptability to soil, climate and poor quality water, as well as acceptability by livestock, indicated that it would be acceptable for the farmers to clear and fence an area in the dune valleys to plant a larger area of *A. halimus*, *A. nummularia* and, perhaps, *C. sturtii* for use as a fodderbank/drought reserve.

<u>Kenhardt, the Nama Karoo</u>: Autumn and spring plantings of the following groups of plants were established on the "Klipkoppies" farm: F_1 seedlings of Hatfield Elite Oldman Saltbush (OMS), *Cassia*, several tree species (in co-operation with the Department of Water Affairs and Forestry) and the standard drought tolerant crops, *Opuntia* and *Agave*, *Tripteris* and *Sutherlandia*. Soil conditions at the Klipkoppies farm in Kenhardt were extremely poor (solid lime at a depth of 20 –50 cm overlain by a light sandy soil), but the watering regime was good. Situated close to the homestead, these plantings were well supervised and less browsed by other domestic and wild animals.

<u>Lovedale Farm, Pofadder, in Bushmanland</u>: An evaluation was carried out of *Cassia* and *A. nummularia* planted at two dates and compared to two indigenous shrubs (*Sutherlandia* and *Tripteris*), several tree species and two drought

tolerant fodder crops (*Opuntia* and *Agave*). Herbivory had a particularly heavy impact on the indigenous shrubs, with only light browsing on *Cassia* and very little utilization of saltbush. Of the trees, the Peppercorn was the most successful with moderate success for the *Rhus* and *Gleditsia*. The drought tolerant succulents, *Opuntia* and *Agave*, hold particular promise as drought fodder reserves, as there was very little evidence of herbivory. The observation trial with Devils Claw on this site yielded no results, as none of the plants survived. *Boscia* was planted out at the Witrand Site.

In the Cemetery Paddock at Lovedale, a trial addressed seed and transplant establishment of *Atriplex* and *Cassia* in interrupted rip-lines. These exotics were not as drought hardy as the *Zizyphus* or *Rhus*, which are addressed below in a separate trial, but a fair survival was registered from both plantings made in spring and autumn of this year, under conditions where plants were protected. Where the protective brush packs had been removed, or not applied, the saltbush and *Cassia* seedlings were eaten off at ground level. Thus, transplants need initial watering, water harvest and protection if they are to survive. Unlike seedlings, there has been no establishment from seed, whether treated, to enhance germination, or untreated, and at best will be episodic under natural conditions.

A spring establishment of two indigenous tree species, *Rhus* and *Ziziphus*, was made on bare areas in the Cemetery Paddock, to evaluate two planting dates and such interventions as water-harvesting, stone mulches, water absorbent and thorn branches to protect plants against herbivory. In addition a product (DRI-WATER) allegedly used to good effect on mineland re-vegetation projects in the USA and SA is also being evaluated. The trees were watered on three occasions during the 2002/2003 drought. With the exception of the DRI-WATER treatments (which were uprooted by porcupines), trees survived very well. Herbivory remained a problem especially on unprotected plants; even with brush packs shoots that emerged from the protective canopies were browsed. Therefore, protective fencing was erected around this site.

A “piosphere” trial was established close to the “Mostert Paddock” in Lovedale, where basin catchments, located at different distances from the water trough, were seeded with indigenous shrub species and protected with branches of “three-thorn” (an invasive indigenous species). Although germination in the 2001/2002 season was fair to excellent with a gradient along the transect, the extreme conditions experienced in 2002/2003 resulted in a very high mortality.

The “New Site” (adjacent to tracks running through the cemetery paddock and east of the Quiver Tree Site) was seeded with indigenous species into rip-lines in bare areas and protected by three-thorn branches, similar to the planting at the Quiver Tree site. Germination was good in 2001/2002. Where the un-anchored protective branches were blown away, seedlings were grazed by insects and indigenous herbivores, whereas, the mortality of seedlings on protected areas could be ascribed to the severe water stress. It would appear that discontinuous rip lines, which result in greater accumulation of litter and seed and protection against herbivory, are to be recommended over continuous rip lines without protection from herbivory.

<u>Kammieskroon, in Namaqualand</u>: In this winter rainfall area, two sites, “Pendoornhoek”, which receives about 200 mm, and ”Olienfontein”, which receives about 400 mm, were used. Olienfontein is cooler and moister, while Pendoornhoek, only 20 km distant, is at a lower altitude and is much drier. Each site was planted in the spring of 2002 and in the autumn of 2003 season with Hatfield Elite OMS and *Cassia*, as well as with indigenous shrubs such as *Sutherlandia* and *Tripteris* and exotic winter rainfall species such as Tagasaste (*Chaemecytis*), Olive and carob from the Mediterranean and *Boscia* from the Northern Cape. By the spring of 2003 the autumn planting of OMS, *Cassia*, *Tripteris* and *Sutherlandia* as well as temperate legumes such as *Teline* and *Chaemacytis* had been affected by poor conditions (drought compounded by heavy browsing), resulting in a high mortality at both sites. With respect to the plants that survived from the spring planting as well as the plants of the more successful autumn planting, the OMS at Pendoringhoek had almost been destroyed by browsing while there was relatively little damage at Olienfontein. *Cassia* was very lightly browsed at both sites.

E. 2 Nutritive value and composition of fodder shrubs (South Africa)

E.2.1. Comparison of chemical composition of Atriplex spp. grown under South African conditions with regard to site, species and plant parts: This study determined seasonal changes in chemical composition of leaves and stems of different *Atriplex* spp. and difference in quality between localities in semi-arid areas.

Materials and Methods

Mature *Atriplex* species were selected for this study from three sites during March and July 2002: Hatfield, Pretoria (Gauteng), Mier, (Northern Cape) and Lovedale (Northern Cape). Samples collected from each species consisted of small branches with stems not larger than 5 mm in diameter. Mature leaves and twigs were collected from *A. nummularia*, *A. canescens* (Santa Rita) and *A. canescens* (Veld Reserve I). After collection the samples were dried at 60 °C for 48 hours, the leaves separated from the stems and then ground through a 1-mm screen using a mill. By weighing the leaves and stems, a leaf to stem ratio was calculated. Organic matter digestibility (IVOMD) was estimated by the *in vitro* method described by Tilley & Terry (1963) as modified by Engels & Van der Merwe (1967). Crude protein (CP) concentration was determined by Kjeldahl (AOAC, 2000). Analyses of variance with the Proc GLM model (SAS, 1994) were used to determine the significance between different species of *Atriplex* in different locations, seasons and plant parts. Means and standard deviations (s.d.) were calculated and significance (5%) among means was determined by Bonferroni's test (Samuels, 1989).

Results and Discussion

The crude protein, IVOMD and leaf to stem ratio of the three *Atriplex* spp. collected at three different sites are presented in Table 1. The CP concentration varied from 93.8 g/kg in *A. canescens* (Veld Reserve 1) to 194.6 g/kg in *A. nummularia* (Mier). The CP values correspond well with those reported by Senock *et al.* (1991) for *A. canescens.* The CP concentration for all the species was the highest at Mier. In most cases there was a significant difference in the CP concentration for a specific species between sites. *Atriplex nummularia* had the highest CP concentration at all the studied localities. Except for *A. nummularia* there were no significant differences in the IVOMD values for different localities. The IVOMD values for *A. nummularia* were in all cases higher (P < 0.05) than the other species. Significant differences were observed for leaf to stem ratios for different species and different sites. Except for *A. nummularia*, significant differences were observed between March (summer) and July (winter) with respect to the CP and IVOMD concentration of the species concerned (Table 2).

Table 1 Differences in the mean (± s.d.) chemical composition (g /kg DM) and leaf to stem ratio of three *Atriplex* spp. between localities and species

Species	Variable	Location		
		Hatfield	Mier	Lovedale
A. canescens (Santa Rita)	CP	110^{a}_{1} (± 12)	143^{b}_{1} (± 14)	126^{a}_{1} (± 9.6)
	IVOMD	467^{a}_{1} (± 22)	496^{a}_{1} (± 10)	474^{a}_{1} (± 21)
	leaf to stem ratio	53.49^{a}_{1} (± 2.1)	76.03^{b}_{1} (± 2.4)	59.60^{a}_{1} (± 3.6)
A. canescens (Veld Reserve 1)	CP	94^{a}_{1} (± 11)	149^{b}_{1} (± 14)	119^{c}_{1} (± 10.1)
	IVOMD	463^{a}_{1} (± 24)	503^{a}_{1} (± 18)	471^{a}_{1} (± 26)
	leaf to stem ratio	35.92^{a}_{2} (± 1.8)	56.55^{b}_{2} (± 1.9)	44.05^{ab}_{2} (± 2.4)
A. nummularia	CP	124^{a}_{2} (± 11)	195^{b}_{2} (± 13)	183^{b}_{2} (± 14)
	IVOMD	487^{a}_{1} (± 26)	599^{b}_{2} (± 21)	591^{b}_{2} (± 25)
	leaf to stem ratio	54.13^{a}_{1} (± 1.4)	68.45^{b}_{12} (± 1.3)	70.17^{b}_{1} (± 1.5)

Row (abc) and Column (12) means with common superscripts do not differ (P > 0.05)
CP – crude protein; IVOMD – *In vitro* organic matter digestibility

CP concentration of *A. nummularia* differed significantly from the other species in both seasons but the concentration of *A. canescens* (Santa Rita) and *A. canescens* (Veld Reserve 1) did not differ from each other. The IVOMD of *A. nummularia* did not show significant differences compared to the other species with respect to seasonal trends and only the leaf to stem ratio of *A. canescens* (Veld Reserve1) showed significant differences in the two seasons. In addition, the stem to leaf ratio of the species concerned also differed significantly. The same trend was reported by Sparks (2003).

Table 2 Differences in the mean (± s.d.) chemical composition (g /kg DM) and leaf to stem ratio of three *Atriplex* spp. between species and seasons

Species	Variable	Seasons	
		March (Summer)	July (Winter)
A. canescens (Santa Rita)	CP	136^{a}_{1} (±11)	119^{b}_{1} (±6)
	IVOMD	515^{a}_{1} (±12)	443^{b}_{1} (±13)
	leaf to stem ratio	65.93^{a}_{1} (±2.1)	60.15^{a}_{1} (2.6)
A. canescens (Veld Reserve 1)	CP	129^{a}_{1} (±3)	112^{b}_{1} (±6)
	IVOMD	506^{a}_{1} (±16)	452^{b}_{1} (±14)
	leaf to stem ratio	54.75^{a}_{2} (±3.6)	36.27^{b}_{2} (2.9)
A. nummularia	CP	172^{a}_{2} (±11)	163^{a}_{2} (±9)
	IVOMD	562^{a}_{2} (±14)	556^{a}_{2} (±11)
	leaf to stem ratio	63.99^{a}_{1} (±2.0)	64.50^{a}_{1} (±2.2)

Row (abc) and Column (12) means with common superscripts do not differ (P > 0.05)
CP – crude protein; IVOMD – *In vitro* organic matter digestibility

CP values for the leaves were in all cases higher (P < 0.05) than the stems (Table 3). Lleaf and stem CP-values of *A. nummularia* were higher (P < 0 05) than the other species. Leaf IVOMD of all species was higher (P < 0.05) than that of the corresponding stems. In addition the IVOMD of the leaves and stems of *A. nummularia* were higher than the other species studied. Peterson *et al*. (1987) noted the same trend for fourwing saltbush.

Table 3 Differences in the mean (± s.d.) chemical composition (g /kg DM) of three *Atriplex* spp. between species and plant parts

Species		Plant part	
		Leaves	Stems
A. canescens (Santa Rita)	CP	171^{a}_{1} (± 18)	82^{b}_{1} (± 12)
	IVOMD	673^{a}_{1} (± 18)	285^{b}_{1} (± 36)
A. canescens (Veld Reserve 1)	CP	169^{a}_{1} (± 16)	73^{b}_{1} (± 16)
	IVOMD	705^{a}_{1} (± 15)	233^{b}_{1} (± 48)
A. nummularia	CP	217^{a}_{2} (± 14)	117^{b}_{2} (± 16)
	IVOMD	732^{a}_{12} (± 30)	386^{b}_{2} (± 21)

Row (abc) and Column (12) means with common superscripts do not differ (P > 0.05)
CP – crude protein; IVOMD – *In vitro* organic matter digestibility

Conclusions: Differences due to season and location of the three species, demonstrated the importance of these factors when planning fodder budgets.

E.2.2 A comparison of Cassia sturtii, Tripteris sinuatum and Sutherlandia microphylla: three fodder shrubs applicable to revegetation of degraded rangeland in the Northern Cape Province: This trial included one exotic (*Cassia sturtii*) and two indigenous (*Sutherlandia microphylla* and *Tripteris sinuatum*) species. It compared the three species, over time, in terms of production, leafiness and certain qualitative characteristics.

Materials and Methods

Twenty replicates per species were randomly allocated to plots. Five seedlings of a species were planted per plot. Four replicates per species were harvested (20 cm above ground level) randomly at each harvest date (7th July, 18th August, 29th September, 10th November and 22nd December 2003). The plant material was separated into leaf and stem material and then dried in a forced draught oven at 60 °C for 24 hours. Plant production was based on dry matter (DM) yields. The percentage leaf material was also determined. Representative samples of the final harvest were analysed for *in vitro* digestible organic matter (IVDOM %) (Tilley & Terry, 1963), as modified by Engels & Van der Merwe (1967), crude protein (CP) (AOAC, 2000) and ash (AOAC, 2000).

An analysis of variance with the GLM model (SAS, 1994) was used to determine the significance of differences among species, leaves and stems and harvest dates. Means and standard deviations (s.d.) were calculated and significance ($P < 0.05$) among means was determined by the Bonferroni test (Samuels, 1989).

Results and Discussion

Sutherlandia had the highest DM yield (Figure 1). After the third harvest *Sutherlandia* exhibited a drastic increase in yield in comparison with the other species. Severe frost (experienced at the end of August 2003) affected both *Tripteris* and *Cassia*. *Cassia*, although affected by the frost, recovered quickly and an increase in yield was noted after 29th September. Although *Tripteris* produced more material in the initial harvests, the frost took its toll and recovery was slower than that of C*assia*. *Cassia* had a slow start and the lowest DM yield over time. By the 22nd of December production levels of *Cassia* were equivalent to those of *Tripteris.* There was also an increase in the amount of weeds in the camp and they too, seemed to impact the growth of *Tripteris*.

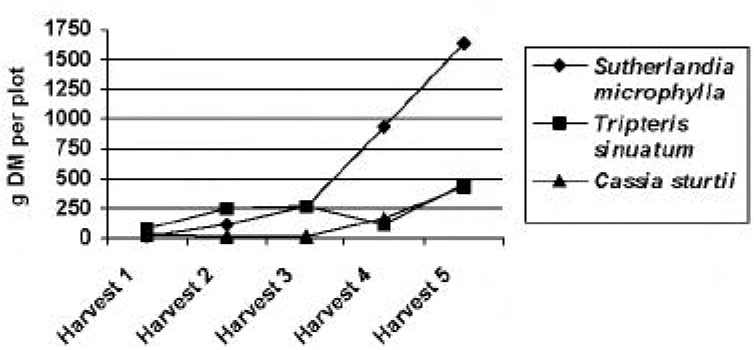

Figure 1 The dry matter (DM) production (g per plot) of different fodder shrub species at different harvest times

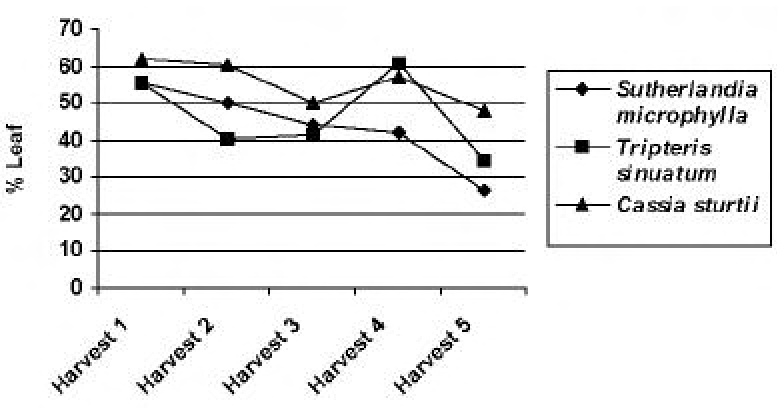

Figure 2 The percentage leaf in different species at different harvest dates

As the plants increased in size, a decrease in the percentage leaf material was observed (Figure 2). Of the three species the largest decrease was observed in *Sutherlandia*. *Cassia* had the highest percentage leaf. The drastic decrease in percentage leaf between the November and December harvests in *Tripteris* may have been due to a heavy weed infestation at that stage.

Harvested material was analysed for *in vitro* digestible organic matter (IVDOM), ash and CP concentrations (Table 1). The leaves of *Tripteris* and *Sutherlandia* had the highest CP concentrations and IVDOM ($P < 0.05$). *Cassia* stems had higher IVDOM values than those of both *Tripteris* and *Sutherlandia* ($P < 0.05$), but no difference was observed in the CP concentration among the different species ($P > 0.05$). Research conducted by Sparks (2003) indicated that *Cassia* was nutritionally inferior to *Atriplex nummularia*. The leaves of *Tripteris* had a higher percentage ash than leaves of both

Sutherlandia and *Cassia* ($P < 0.05$). In all species higher IVDOM and CP concentrations were observed in the leaves than in the stems ($P < 0.05$). The NRC (1981) suggested that the protein requirement for maintenance of a 50 kg doe is 75 g/kg feed. All three species met this requirement.

Table 1 Comparison of the *in vitro* digestible organic matter (IVDOM) and the ash and crude protein (CP) concentrations of the leaves and stems of different fodder shrub species (± s.d.)

	Stems = 3 mm	Leaves
Sutherlandia microphylla		
IVDOM, %	38.9^{ab}_{1} (± 2.7)	66.0^{b}_{2} (± 3.7)
Ash, g/kg DM	25^{a}_{1} (± 3.0)	64^{a}_{2} (± 2.0)
CP, g/kg DM	88^{a}_{1} (± 9.0)	225^{b}_{2} (± 19)
Tripteris sinuatum		
IVDOM, %	33.0^{a}_{1} (± 4.9)	66.8^{b}_{2} (± 1.2)
Ash, g/kg DM	105^{c}_{1} (± 14)	184^{b}_{2} (± 17)
CP, g/kg DM	98^{a}_{1} (± 8)	216^{b}_{2} (± 47)
Cassia sturtii		
IVDOM, %	41.8^{b}_{1} (± 2.4)	55.4^{a}_{2} (± 2.3)
Ash, g/kg DM	53^{b}_{1} (± 2)	73^{a}_{2} (± 10)
CP, g/kg DM	76^{a}_{1} (± 10)	147^{a}_{2} (± 11)

^{ab}Column means with common superscripts do not differ; ^{1,2}Rows means with common subscripts do not differ ($P > 0.05$)

Conclusions: Both indigenous species have potential as fodder shrubs for revegetation projects. Although the establishment of such fodder shrubs is often not financially feasible for small scale farmers (Le Houérou, 2000), it is important that farming systems be used which are based on sustainable practices in order to restore degraded areas and maintain them at a satisfactory production level.

E.2.3. Qualitative characteristics of some Atriplex species and Cassia sturtii at two sites: This study evaluated the interspecies and location variation in chemical composition of certain qualitative parameters between *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii* grown in two different locations.

Materials and Methods

Leaves were collected from two experimental sites differing in ecological conditions of *Atriplex canescens*, *A. halimus*, *A. nummularia* and *Cassia sturtii*. Site one was at the Experimental Farm of the University of Pretoria, Gauteng. It is a summer rainfall area with a precipitation of 650 mm per annum. The soil type is a Hutton form (MacVicar *et al*., 1977), well drained, slightly acidic and consists of a good nutrient status. The Hutton type is a deep clay-loam soil with approximately 25% clay and an effective depth of 600 mm+. According to soil analysis, the soil pH(H2O) was 5.7, P, K, Ca, Mg and Na status were 25, 200, 800, 400 and 40 mg/kg, respectively. Site two was at the farm Lovedale in the Kenhardt district, Northern Cape province. It is a summer rainfall area with an average annual rainfall of approximately 130 mm. According to MacVicar *et al*. (1977), the soil type is also a Hutton form, slightly alkaline and consists of a good nutrient status (pH(H2O) 8.4, P, K, Ca, Mg and Na status of 14, 337, 3445, 136 and 179, respectively). This type is a shallow calcareous sandy soil with less than 10% clay and an effective depth of not more than 300 mm. Sample material randomly collected for each species on both sites was from approximately five year old plants. Samples of each plant of the same species in each replication were kept apart and not pooled.

Samples were dried in a force draught oven for 24 hours at 60 °C and milled through a 1 mm screen of a Beaver mill for chemical analysis to determine qualitative measurement. Crude protein (CP) and ash concentrations were determined according to AOAC (2000) and neutral detergent fibre (NDF) and acid detergent lignin (ADL) concentrations according to the method of Van Soest & Wine (1967). *In vitro* digestible organic matter (IVDOM) was done according to the method of Tilley & Terry (1963) as modified by Engels & Van der Merwe (1967). A model was tested for each of the dependant variables. An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between species, locations and first order interactions for the dependant variables. The level of significance between least square means was tested with the help of the Bonferroni's test according to Samuels (1989).

Results and Discussion

Significant differences among the three *Atriplex* spp. and *C. sturtii* with respect to the nutritive value were evident, and also between the species at Hatfield and Lovedale (Table 1). A number of authors also reported high CP values for *A. nummularia*, as in this study (Smit & Jacobs, 1978; Khalil *et al.*, 1986; Malan, 2000). According to Welch & Monsen (1981), genetic variation plays an important role in the protein concentration in *Atriplex* spp., while season and soil fertility will also have a major effect on CP concentration (McArthur *et al*., 1981). It has to be kept in mind that up to 60% of the CP fraction in plants, may be non protein nitrogen (Benjamin *et al*., 1992).

Table 1 The crude protein (CP), *in vitro* digestible organic matter (IVDOM), neutral detergent fibre (NDF) and acid detergent lignin (ADL) concentrations (g /kg DM) of leaf material for *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii* at two different locations (hand cut samples)

		Species			
Location	Parameter	*A. canescens*	*A. halimus*	*A. nummularia*	*Cassia sturtii*
Hatfield	CP	176_1^a (± 22)*	187_1^a (± 48)	208_1^a (± 10)	147_1^a (± 31)
	IVDOM	738_1^b (± 9)	718_1^b (± 45)	738_1^b (± 19)	574_1^a (± 65)
	NDF	378_2^c (± 9)	328_2^b (± 4)	407_2^c (± 13)	250_1^a (± 25)
	ADL	139_2^b (± 6)	131_1^b (± 9)	138_1^b (± 12)	75_1^a (± 5)
Lovedale	CP	198_1^b (± 7)	206_1^a (± 38)	234_1^b (± 10)	114_1^a (± 26)
	IVDOM	716_1^b (± 20)	773_1^b (± 4)	757_1^b (± 42)	529_1^a (± 88)
	NDF	295_1^b (± 6)	297_1^b (± 20)	332_1^b (± 10)	223_1^a (± 23)
	ADL	98_1^b (± 1)	131_1^c (± 3)	137_1^c (± 2)	71_1^a (± 06)

Row (abc) and Column (12) means with common superscripts do not differ ($P > 0.05$); *(Standard deviation)

Due to significant interactions, no pooled results for site comparison are presented. No significant differences in IVDOM occurred between the *Atriplex* spp. at both sites as well as between sites. *Cassia sturtii* had significantly lower IVDOM and NDF values than the *Atriplex* spp. The values of Malan (2000) for *Atriplex* spp. supported these results. The IVDOM range of all the plants at both sites fell within the range (and even above) (up to 690 g/kg) of *in vitro* DM digestibility noted for tropical browse plants (Sawe *et al*., 1998) and *in vivo* OM digestibilities in goats (Kibria *et al*., 1994). As NDF is more closely associated with intake then digestibility (Meissner *et al*., 1989) one can conclude from the relatively low NDF values of the leaves of both *Atriplex* spp. and *C. sturtii*, that fairly high intakes by small stock should be possible. The ADL concentrations of the *Atriplex* spp. at both sites were significantly higher than those of *C. sturtii*. Only *A. canescens* differed significantly in terms of ADL concentration between the two sites. Acid detergent lignin values of 145 g/kg reported by Kaitho *et al.* (1998) for *A. halimus* agreed with those reported in this experiment for the *Atriplex* spp. Lower values for *A. nummalaria* (93 g/kg) were reported by Abou El Nasr *et al.* (1996).

Conclusions: All the species evaluated in this experiment proved to have a fair potential as fodder crops, with high CP and IVDOM concentrations as well as fairly low NDF.

E.2.4. Qualitative characteristics of Boscia foetida at different sites: This study examined the suitability of *Boscia foetida* as a fodder source for goats.

Materials and Methods

Nine samples (trees) of edible material (twigs up to 4 mm in diameter, and leaves) of *Boscia foetida* were collected at three sites in South Africa during February 2003. Samples were taken from mature trees. The sites were Kenhardt, Northern Cape Province, at an altitude of 789 m with an average rainfall of 155 mm; Klein Pella, Northern Cape Province, at an altitude of 836 m with an average rainfall of 89 mm; and Marken, Limpopo Province, at an altitude of 849 m with an average rainfall of 445 mm. The samples were air dried, leaves and stems were separated, milled and then analysed for the following: *In vitro* digestible organic matter (IVDOM) according to the method of Tilly & Terry (1963) as modified by Engels & Van der Merwe (1967), crude protein (CP) and macro and trace minerals (AOAC, 2000), neutral detergent fibre (NDF) (Robertson & Van Soest, 1981) and acid detergent lignin (ADL) (Goering & Van Soest, 1970). An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between different areas, leaves and stems. Least square means and standard deviations (s.d.) were calculated. Significance of difference (5%) between means was determined by multiple comparisons using Bonferroni's test (Samuels, 1989).

Results and Discussion

The CP concentration of stems varied from 96 g/kg at Kenhardt to 150 g/kg at Marken ($P < 0.05$). The highest CP concentration for leaves (187 g/kg) was recorded at Klein Pella and it was significantly higher than the CP concentration of leaves collected at Kenhardt (133 g/kg). The CP concentration of *Combretum* spp. foliage ranged from 62 g to 125 g/kg DM and that of *Colophospermum mopane* from 99 to 169 g/kg DM (Lukhele & Van Ryssen, 2003). These compared favourably with that of the reported *Boscia* leaves. Neutral detergent fibre concentrations in *B. foetida* leaves were higher than the range reported by Lukhele & Van Ryssen (2003) for *Combretum* spp. (279 g – 409 g/kg DM) while the ADL concentrations in *B. foetida* leaves were also higher than the reported values of Lukhele & Van Ryssen (2003) for the *Combretum* spp. and *C. mopane*. High NDF and ADL values of *B. foetida* will most probably have a negative influence on digestibility and intake of such material by grazing herbivores. The higher IVDOM values of the *Boscia* leaves, in comparison to stems, are important. It is reported that leaves have a shorter rumen retention time compared to stems (Minson, 1982), which may permit more dry matter to be consumed if mainly leaves are browsed. According to Tetthen, (1974), as cited by NRC (1981), goats are selective feeders and will select leaf material before browsing on stems of a specific fodder. The Ca, P, Mg, Cu, Zn and Mn concentrations are presented in Table 2.

Table 1 Mean (± s.d.) chemical composition of *Boscia foetida* at different sites (g/kg) DM

Site		CP	NDF	ADL	IVDOM
Marken	Leaf	183^{a} (46.3)	507^{b} (63.6)	168^{a} (10.4)	458^{a} (4.8)
	Stem	150_{2} (17.0)	760_{2} (15.5)	222_{1} (10.5)	215_{1} (1.6)
Kenhardt	Leaf	133^{b} (6.0)	575^{b} (17.1)	136^{a} (9.6)	462^{a} (1.2)
	Stem	96_{1} (8.8)	648_{1} (7.1)	159_{1} (7.5)	228_{1} (3.2)
Pella	Leaf	187^{a} (6.8)	536^{b} (67.4)	153^{a} (4.0)	479^{a} (5.5)
	Stem	109_{1} (11.5)	641_{1} (11.5)	201_{1} (40.5)	203_{1} (4.1)

Row (ab) and Column (12) means with common superscripts do not differ ($P > 0.05$)
CP - crude protein; NDF - neutral detergent fibre; ADL - acid detergent lignin;IVDOM - *in vitro* digestible organic matter

Table 2 Mean (± s.d.) macro-mineral (g/kg DM) and trace mineral (mg/kg DM) concentrations of *Boscia foetida* at three sites

		Ca	P	Mg	Cu	Zn	Mn
Marken	leaf	5.5^{a} (2.1)	0.89^{a} (0.4)	3.8^{a} (0.3)	9.8^{a} (5.0)	13.0^{a} (1.7)	114^{a} (13.8)
	stem	3.2_{1} (0.8)	0.60_{1} (0.2)	0.9_{1} (0.1)	8.2_{1} (1.7)	13.4_{1} (3.2)	29.3_{1} (8.3)
Kenhardt	leaf	6.2^{a} (0.1)	0.56^{a} (0.1)	1.0^{b} (0.1)	3.0^{b} (0.1)	15.3^{a} (4.0)	133^{a} (14.4)
	stem	6.3_{2} (0.2)	0.63_{1} (0.1)	1.0_{1} (0.1)	3.0_{2} (0.1)	36.3_{2} (8.1)	35.0_{1} (7.0)
Pella	leaf	6.0^{a} (1.9)	0.83^{a} (0.1)	2.7^{a} (0.1)	5.7^{b} (1.9)	24.0^{b} (11.1)	78^{b} (11.0)
	stem	5.1_{2} (1.0)	0.71_{1} (0.2)	1.0_{1} (0.2)	4.9_{2} (0.6)	42.0_{2} (15.1)	22.0_{1} (12.2)

Column for leaves (a,b) and stems (1,2) means with common superscripts do not differ significantly ($P > 0.05$)

The Ca, P and Mg concentrations of *B. foetida* were generally higher in leaves than in stems. Although the Ca concentrations were lower than those reported for *B. albitrunca* (11.1 - 14.4 g/kg) by Groenewald *et al.* (1967), Ca and Mg concentrations of *B. foetida* foliage were still higher than growth requirements of goats (Underwood, 1981; AFRC, 1998). The P concentrations were, however, too low (AFRC, 1998). *Boscia foetida* leaves contained sufficient Cu concentrations at Marken for maintenance requirements of goats, but not at Klein Pella and Kenhardt (AFRC, 1998). The Mn concentrations at all sites were sufficient to meet maintenance requirements, but not that of Zn (AFRC, 1998).

Conclusions: Plant leaves had an acceptable level of digestibility. *Boscia foetida* contains sufficient concentrations of Ca, Mg and Mn for the requirements of goats but lacks in P, Cu and Zn. The wide Ca:P ratio may present a problem, but ruminants can tolerate a relatively wide Ca:P ratio in the diet, provided that the P intake is high (Underwood, 1981).

E.2.5. Carbohydrate supplementation on intake and digestibility of Atriplex nummularia: This study examined the influence of carbohydrate supplementation on the digestibility of *A. nummularia* in sheep.

Materials and Methods

Ten rumen cannulated sheep were used in a split plot design. The experimental diets consisted of *A. nummularia* supplemented with maize (medium fermentability) and barley (high fermentability) at three different levels, 15%, 30% and 45% on a dry matter basis. Neutral detergent fibre (NDF) concentrations of the diets were determined by the method described by Van Soest & Wine (1967). An electronic pH meter was used to determine the pH of rumen fluid after each sample was taken (Robinson *et al.*, 1986). An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between treatments and different levels of supplementation. Least square means and standard errors (SE) were determined and significance (5%) between least square means was determined by using Bonferroni's test (Samuels, 1989).

Results and Discussion

The results in Table 1 indicate that supplementation of both energy sources tended towards to increase (not significant) dry matter intake. Significant differences occurred at the 45% of maize and 30% of barley inclusion levels. The higher intakes could have been as a result of the higher palatability and digestibility of the two energy sources. The NDF digestibility tended towards a significant increase from 0% to 15% and from 0% to 30% and 45%. These increases may be due to positive associative effects in the rumen. Significant positive associative effects of grain supplementation were noted for feedlot animals by Huck *et al*. (1998). Fermentation of starch in the rumen increased propionic acid concentrations in the rumen and improved starch utilization. It also appeared to increase uptake of amino acids from the small intestine (Theurer *et al*., 1999). This may be partly explained by the higher intake with energy supplementation. With the 30% and 45% supplementation of both energy sources, there was a non-significant drop in the NDF digestibility, most probably due to negative associative effects in the rumen. There was a significant drop in rumen pH at 30% and 45% supplementation in both energy sources. The drop in pH supported the possibility of negative associative effects in the rumen. These negative associative effects were most probably responsible for the decrease in NDF digestibility when the supplementation was raised from 15% to 30% and from 30% to 45% in both treatments. The lower intake of barley at the 45% supplementation level (high fermentation rate), together with a drop in rumen pH, was related to the lower NDF digestibility. A lower ruminal pH has a negative effect on cell wall digestibility and thus on intake (Minson, 1990). Differences between the two carbohydrate sources were small and not significant, except for intake at the 45% supplementation level. McCarthy *et al*. (1989) reported positive intake results in favour of the slower fermentable carbohydrate source, which was the case with the maize supplementation.

Table 1 Dry matter intake, % NDF digestibility and rumen pH, of sheep fed *Atriplex nummularia* at different levels of supplementation by two energy sources

		Treatments	
	Supplementation level (%)	Maize	Barley
Intake /kg W (g /day)	0	23.46_1^a (± 3.7)*	32.2_1^{ab} (± 3.3)
	15	23.58_1^a (± 3.3)	21.2_1^a (± 3.7)
	30	33.51_1^{ab} (± 3.3)	37.9_1^b (± 3.3)
	45	38.63_1^b (± 3.3)	25.7_2^a (± 3.7)
% NDF digestibility	0	30.24_1^a (6.5)	28.21_1^a (5.8)
	15	51.58_1^a (5,8)	61.57_1^b (6.5)
	30	40.22_1^a (5.8)	51.54_1^b (5.8)
	45	42.72_1^a (5.8)	41.56_1^{ab} (6.56)
pH (H_2O)	0	6.98_1^a (± 0.11)	7.05_1^a (± 0.10)
	15	6.77_1^{ab} (± 0.10)	6.75_1^{ab} (± 0.11)
	30	6.50_1^{bc} (± 0.11)	6.53_1^b (± 0.10)
	45	6.10_1^c (± 0.10)	5.94_1^c (± 0.10)

Column (a,b,c) and row (1,2) means with common scripts do not differ (P > 0.05)
*Values in brackets designate standard errors

Conclusions: Supplementation of *A. nummularia* with an energy source tended to increase intake. The tendency of energy sources to increase NDF digestibility diminished when the supplemental level was raised from 15% to 30% and from 30% to 45%. These results suggested that barley and maize supplemented at a level of 15% gave the highest incremental increase in DM and NDF digestibility in *A. nummularia*.

E.2.6 Interspecies and location variation in oxalic acid concentrations in certain Atriplex species and Cassia sturtii: Chronic oxalate poisoning in herbivores has a depressing effect on animal production. The aim of this study was to evaluate the interspecies variation in oxalic acid (OA) concentrations in leaves between *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii*.

Materials and Methods

Leaves were collected from two experimental sites different in ecological conditions. Site one was at the Experimental Farm of the University of Pretoria, Gauteng, at an altitude of 1360 m. It is a summer rainfall area with an average precipitation of 650 mm per annum. The soil type is a Hutton form (MacVicar *et al*., 1977), well drained, slightly acidic and consists of a good nutrient status and an effective depth of 600 mm+. According to soil analysis, the soil pH(H2O) was 5.7, P status 25 mg/kg, K status 200 mg/kg while the Ca, Mg and Na status were 800, 400 and 440 mg/kg respectively. Site two was at the farm Lovedale in the Kenhardt district, Northern Cape province, at an altitude of 1015 m. It is a summer rainfall area with an average annual rainfall of approximately 130mm. The soil is also a Hutton form, slightly alkaline and consists of a good nutrient status. According to soil analysis, the soil pH(H2O) was 8.4, P status 14 mg/kg, K status 337 mg/kg, while Ca, Mg and Na status were 3445, 136 and 179 mg/kg respectively. This type is a shallow calcareous sandy soil with less than 10% clay and an effective depth of not more than 300 mm. Leaves were collected from *Atriplex canescens*, *Atriplex halimus*, *Atriplex nummularia* and *Cassia sturtii*. Sample material randomly collected for each species on both sites was from approximately five year old plants. Leaves (mostly mature) were dried in a force draught oven for 24 hours at 60 °C and milled through a 1 mm screen. Oxalic acid concentration was measured through colorimetric determination of OA as oxalyldihyrazidee, as described by Figenschou & Marais (2000) (personal communication, Cedara Agricultural Research Institute, KwaZulu-Natal, South Africa). This method is quite different from the titration method of Moir (1953) for determining total oxalates. Oxalic acid was extracted from 0.5 g of the milled plant material and measured against 10 mL of a standard pre-prepared OA solution. A series of steps were followed until a mixture with a blue colour was formed. A darker blue colour represented a higher oxalate concentration. The absorption of the mixture was read at 600 nm on a COALAB (model dds CP500) colorimetric spectrophotometer. Calcium and Mg concentrations were determined by atomic absorption spectrophotometry and Na and K concentrations by flame photometry (AOAC, 2000). Three samples per species per location were analysed. An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between species, locations and first order interactions for the dependant variables. Significance between least square means was tested with the Bonferroni's test (Samuels 1989).

Results and Discussion

OA concentrations of the three *Atriplex* spp. at Hatfield and Lovedale were higher ($P < 0.05$) than those of *C. sturtii* (Table 1). All species except *A. canescens* had lower ($P < 0.05$) OA concentrations at Hatfield than at Lovedale. Oxalic acid concentrations of 5.8% recorded by Wilson (1966) for *A. nummularia* were higher than values recorded in this study. Perhaps, the author used younger plants during the experiment. OA concentrations recorded in this experiment were not considered to be toxic to grazing livestock (Watson *et al*. 1987).

Table 1 Oxalic acid concentration (%) of leaf material for *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii* at two different locations (DM basis)

Location	Species			
	A. canescens	*A. halimus*	*A. nummularia*	*C. sturtii*
Hatfield	3.30_1^c (± 0.12)*	2.66_1^b (± 0.08)	3.26_1^c (± 0.07)	0.85_1^a (± 0.25)
Lovedale	3.50_1^b (± 0.01)	3.44_2^b (± 0.03)	3.51_2^b (± 0.03)	1.11_2^a (± 0.11)

Row (a,b,c) and column (1,2) means with common scripts do not differ ($P > 0.05$); * Standard deviation

To interpret the results, it is important to determine whether plants are calciophobes or calciotrophes (Wollenweber, 2002 – personal communication, Department of Plant Biology, The Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg, Denmark). Calciophobes are enriched in oxalate as the dominant carboxylate binding Ca2+ contents and resulting in low free vascualar Ca2+, whereas calciotrophes show high total and free Ca2+ contents (Ca2+ : K > 1) and less oxalate. These physiotypes – defining plants according to their eco-physiological properties – are quite constant and reveal information on how species adapt to their environment. According to Wollenweber (2002) – (personal communication), many eco-physiological studies have emphasized site-specific properties of the investigated plants (xero- *vs*. hygrophytes; glyco- *vs*. halophytes; acidophile *vs*. basiphile plants; calcifuge *vs*. calcicole plants). There is, however, a relationship between ecological and physiological aspects of plant metabolism, and certain physiological properties of plants (i.e. enzyme activities, ion ratios and ion balance) may be used for the interpretation of results from ecological studies. These considerations resulted in the formulation of the physiotype concept. A physiotype being a taxonomic unit with defined physiological properties (i.e. the ratio Ca2+ : K+ or organic/inorganic ions – assessable *via* biochemical analysis of plant matter).

To determine the physiotypes of the species used for analysis, the percentages as noted in Table 1 and values determined for Ca, Mg, Na and K, in the study, were converted to micro-equivalents per g dry mass (not taking into account differences in biomass) and are represented in Table 2 and graphically in Figures 1 to 3. From the cation concentration (Fig. 1) it can be seen that the highest cation concentration was in *A. halimus* at both sites, while it was the lowest in *C. sturtii*. Assuming that Ca is mainly bound by oxalate, the following was noted (Fig. 2): The Ca concentration was the highest in *A. canescens* at both sites. Lower values were observed at Lovedale. However, higher oxalate concentrations were noted which lead to low free Ca. *Cassia sturtii* in this case had a high value of free Ca, indicating that this is calciotrophic species. A Ca:K ratio of almost equal to 1, was noted at Hatfield (Fig. 3), while the ratio was < 1 at Lovedale for the *Atriplex* spp. For *C. sturtii* a higher Ca than K concentration was noted, thus a further indication of the calciotrophic nature of *C. sturtii*.

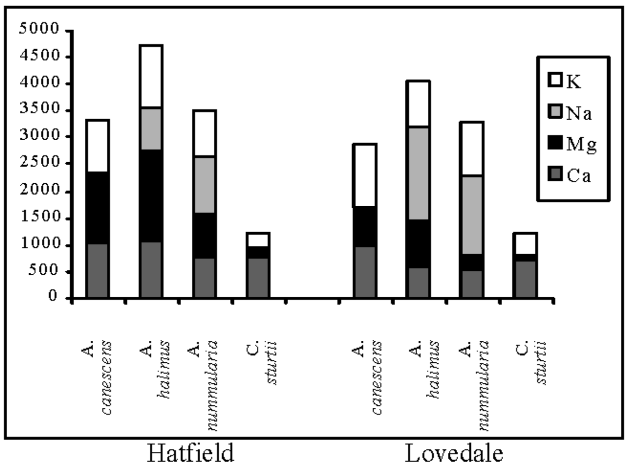

Fig 1 Comparison of cation balances at the two sites

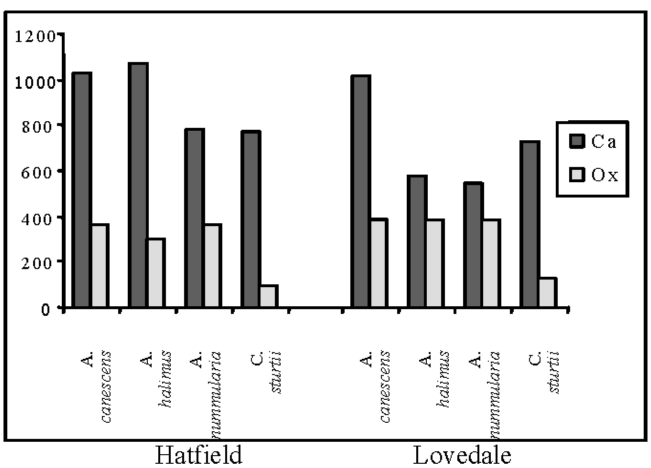

Fig 2 Comparison between Ca and oxalate concentrations

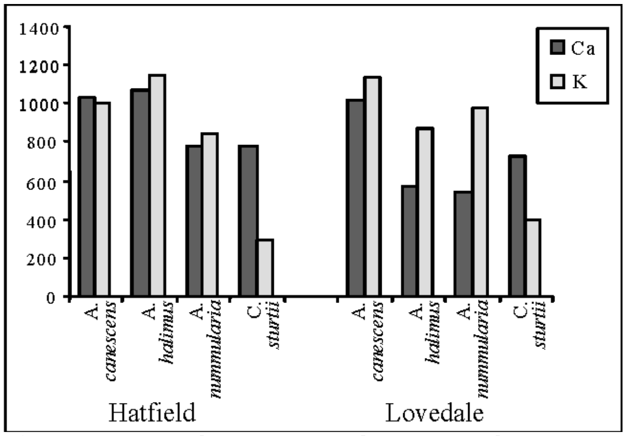

Fig 3 Comparison between Ca and K ratios at the two sites (Units: micro-equivalents per g dry mass)

Table 2 Percentages of Ca, Mg, Na, K and oxalate converted to micro-equivalents per g dry mass

		μeq /g DM					
Location	Species	Ca	Mg	Na	K	Oxalate	K / Ca
Hatfield	*A. canescens*	1 028	1 325	4	997	367	0.97
	A. halimus	1 073	1 670	814	1 154	295	1.08
	A. nummularia	778	798	1 103	844	362	1.08
	C. sturtii	773	165	1	289	94	0.37
Lovedale	*A. canescens*	1 013	683	35	1 133	389	1.12
	A. halimus	574	856	1 755	875	382	1.52
	A. nummularia	544	247	1 513	972	390	1.79
	C. sturtii	724	99	4	402	123	0.55

Conclusions: The high free Ca2+ and lower oxalate concentrations in *C. sturtii* indicates an unlikely possibility of oxalate poisoning in animals grazing this plant. Poisoning may be possible in *Atriplex* spp., especially in young plants where the oxalate levels may play a role irrespective of the Ca:K ratio (Watson *et al*., 1987).

E.2.7. Mineral composition of certain Atriplex species and Cassia sturtii: This study evaluated the interspecies and area variation for macro- and trace mineral concentrations between *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii*.

Materials and Methods

Leaves of *Atriplex canescens*, *A. halimus*, *A. nummularia* and *Cassia sturtii* were collected from two experimental sites differing in ecological conditions. Site one was at the Experimental Farm of the University of Pretoria, Gauteng. It is a summer rainfall area with a precipitation of 650 mm per annum. The soil type is a Hutton form (MacVicar *et al*., 1977), well drained, slightly acidic and consists of a good nutrient status. The Hutton type is a deep clay-loam soil with approximately 25% clay and an effective depth of 600 mm+. According to soil analysis, the soil pH(H2O) was 5.7, the P status and K status were 250 and 200 mg/kg respectively while Ca, Mg and Na status were 800, 400 and 40 mg/kg, respectively. Site two was at the farm Lovedale in the Kenhardt district, Northern Cape province. It is a summer rainfall area with an average annual rainfall of approximately 130 mm. According to MacVicar *et al*. (1977), the soil type is also a Hutton form, slightly alkaline and consists of a good nutrient status (pH(H2O) 8.4, P, K, Ca, Mg and Na status of 14, 337, 3445, 136 and 179 mg/kg, respectively). Sample material randomly collected for each species (three samples per species per site with two replications) was from approximately five year old plants. Samples of each plant of the same species in each replication was kept apart and not pooled. Samples were dried in a force draught oven for 24 hours at 60 °C and milled through a 1 mm screen of a Beaver mill for chemical analysis to determine qualitative characteristics. Atomic absorption spectrophotometry was used to determine Ca, Mg, Zn, Cu and Mn concentrations according to AOAC (2000). Phosphorus concentration was determined colorimetrically (Parkinson & Allen, 1975) and Se concentration by using a hydride generator attached to an atomic absorption spectrophotometer (Perkin-Elmer 2380). A model was tested for each of the dependant variables. An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between species, locations and first order interactions for the dependant variables. Significance among least square means (LSM) was tested with the Bonferroni's test (Samuels 1989).

Results and Discussion

Macro- and trace mineral concentrations are presented in Table 1. *Atriplex halimus* had a higher Ca concentration at Hatfield then at Lovedale (P<0.05), which does not correspond with the very high Ca concentration of the soil at Lovedale. *Atriplex canescens* also had a higher Ca concentration than *A. nummularia* at Lovedale (P<0.05). This contradicts the reports of both Smit & Jacobs (1978) and Khalik *et al.* (1986) who reported higher Ca concentrations in *A. nummularia* than in *A. canescens*. All the species exceeded the Ca requirements for goats (>1.38 g/kg), irrespective of site (NRC, 1981). The P concentration of *A. nummularia* at both sites was higher than the concentrations in the other plants (P<0.05), except for *A. canescens* at Lovedale. Except *A. canescens*, higher P concentrations were noted for all the plants at Hatfield compared to Lovedale (P<0.05). This is in accordance with the higher P soil values of Hatfield. Concentrations of P found in this experiment were higher than the values reported by Khalil *et al.* (1986) for *A. nummularia* and *A. canescens*, but lower than those reported for the same species by Jacobs & Smit (1977). Except for *C. sturtii*, *Atriplex* spp. at Hatfield supplied enough P for maintenance requirements of small stock (>1.6 g/kg) (NRC, 1985). Although ruminants can tolerate a relatively wide Ca:P in the diet (Underwood & Suttle, 1999), the very wide Ca:P ratios in this study (>10:1) may cause a reduction in P bioavailability and a P deficiency could occur (Underwood & Suttle, 1999). The Mg concentrations of both *A. canescens* and *A. halimus* were higher then those of *A. nummularia* and *C. sturtii* at both sites (P<0.05), with higher levels at Hatfield compared to Lovedale (P<0.05), except for *C. sturtii*. This correlates well with the higher Mg soil concentration at Hatfield. According to the NRC (1981) Mg requirements of goats are well below the reported values.

Table 1 Mineral composition of major and trace elements in leaf material of *Atriplex canescens, A. halimus, A. nummularia* and *Cassia sturtii* at two different sites (DM basis)

Site	Minerals	Species			
		A. canescens	*A. halimus*	*A. nummularia*	*Cassia sturtii*
Hatfield	Ca (g/kg)	20.6$_1^a$ (± 4.3)*	21.5$_2^a$ (± 3.7)	15.6$_1^a$ (± 1.9)	15.5$_1^a$ (± 2.3)
	P (g/kg)	1.9$_1^a$ (± 0.2)	1.92$_2^a$ (± 0.3)	2.5$_2^b$ (± 0.3)	1.5$_2^a$ (± 0.1)
	Mg (g/kg)	16.1$_2^c$ (± 3.4)	20.3$_2^c$ (± 4.3)	9.7$_2^b$ (± 1.0)	2.0$_1^a$ (± 0.1)
	Se (µg/kg)	39$_1^a$ (± 20)	22$_1^a$ (± 8)	21$_1^a$ (± 8)	19$_1^a$ (± 4)
	Zn (mg/kg)	110$_2^c$ (± 18)	103$_2^c$ (± 27)	60$_2^b$ (± 15)	22$_1^a$ (± 2)
	Mn (mg/kg)	170$_2^b$ (± 59)	395$_2^c$ (± 49)	153$_2^b$ (± 59)	37$_1^a$ (± 2)
Lovedale	Ca (g/kg)	20.3$_1^b$ (± 1.8)	11.5$_1^{ab}$ (± 2.5)	10.9$_1^a$ (± 3.2)	14.5$_1^{ab}$ (± 1.0)
	P (g/kg)	1.6$_1^{ab}$ (± 0.1)	1.4$_1^a$ (± 0.1)	1.6$_1^b$ (± 0.1)	0.8$_1^a$ (± 0.1)
	Mg (g/kg)	8.3$_1^{bc}$ (± 0.4)	10.4$_1^c$ (± 0.4)	3.0$_1^a$ (± 0.4)	1.2$_1^a$ (± 0.1)
	Se (µg/kg)	257$_2^a$ (± 60)	105$_2^a$ (± 20)	401$_2^a$ (± 180)	314$_2^a$ (± 33)
	Zn (mg/kg)	13$_1^a$ (± 1)	11$_1^a$ (± 2)	14$_1^a$ (± 6)	13$_1^a$ (± 6)
	Mn (mg/kg)	91$_1^a$ (± 8)	116$_1^a$ (± 6)	62$_1^a$ (± 21)	40$_1^a$ (± 6)

Row (abc) and Column (12) means with common superscripts do not differ (P>0.05); *Standard deviation

Although no significant differences in Se concentrations were noted between the different plants at both sites, large differences occurred between the two sites (P<0.05). The Se requirements of small stock are >100 µg/kg DM (NRC, 1985). The concentrations of all the plants at Lovedale will fulfil in these requirements, but not the plants at Hatfield. The concentration of Zn of the different plants at Lovedale did not differ significantly, but differences occurred at Hatfield, where the *Atriplex* spp. had higher values than *C. sturtii* (P<0.05). The Zn concentrations were also higher at Hatfield than at Lovedale (P<0.05), most probably due to the calcareous soils at Lovedale. The inverse relationship between Ca and Zn is well noted (Underwood & Suttle, 1999). The Zn concentrations of plants at Lovedale were marginally above the Zn requirements for goats (10 mg/kg) (NRC, 1981). The Mn concentrations of the *Atriplex* spp. at Hatfield were higher (P<0.05) than the Mn concentration of *C. sturtii*. No differences were noted for Lovedale. Except for *C. sturtii*, differences occurred between the two sites for all the *Atriplex* spp. (P<0.05). The lower Mn concentrations at Lovedale may be due to the high Ca concentration in those soils (McDonald *et al*., 2002). According to the NRC (1985) sufficient Mn was present in all samples to meet the requirements of small stock.

Conclusions: The Ca, Mg, Zn and Mn concentrations were present at sufficient levels to fulfill the requirements of small stock. The P concentration of all plants at Lovedale was marginal in terms of requirements and the Ca:P ratios of all plants may present a problem in terms of P utilization. Supplementation of P should be considered if these plants, irrespective of site, are being utilized by ruminants. Shortages of Se may occur at Hatfield and supplementation could be necessary. The *Atriplex* spp. had considerably higher macro- and trace mineral concentrations at both sites than *C. sturtii*.

E.2.8. Selection (South Africa)

Work on the recurrent selection within the various target species, but especially the *Atriplex nummularia* showed differences within species and selection was made based on nutritrive characteristics. *Cassia* appeared to be very uniform, offering little opportunity for selection within the species. This was, perhaps, due to the single origin of all the material planted at the South African sites from one Israeli site (Omer).

E.2.9. Analyses of plant material (Israel)

Materials and Methods

In vitro organic matter digestibilities and metabolizable energy yields of *Cassia sturtii*, *Atriplex nummularia* and *Atriplex halimus* shrubs were determined. Metabolizable energy yield of the samples was estimated with the Hohenheim Gas method using sheep rumen fluid as the incubating media (Table 1). In this method, the gas produced in anaerobic fermentation of substrate was used to predict the energy value. Rumen liquor and particulate matter were collected before morning feeding from two sheep fed on a roughage diet of mainly poor quality wheat straw and some lucerne hay; the liquor was homogenized, strained and filtered through glass wool. Incubation media was prepared as described by Menke et al. (1979). Samples, each of 200 mg DM, were incubated in triplicate in 100 ml calibrated glass syringes in which 30 ml of the incubation media was added. The glassware was kept at 39°C and flushed with CO_2 before use and the mixture was continually stirred under CO_2 at 39°C. Gas production, as determined by piston movement, was measured over 24 h after correcting for gas production due to rumen fluid alone.

The following equations were used to estimate organic matter digestibility (OMD) and metabolizable energy (ME) of the shrubs:

OMD (%) = 24.91 + 0.722 GP + 0.0815 CP, and

ME (kJ/g DM) = 2.20 + 0.136 GP + 0.0057 CP

where gas production (GP) is in ml/200g dry matter for 24 hours and crude protein (CP) is in g/kg DM.

Results and Discussion

In vitro organic matter digestibilities of the *Cassia sturtii* leaves averaged 38.8% and of the small branches 37.5%, while metabolizable energy yield of the leaves averaged 4.23 kJ/g DM and of the small branches averaged 4.16 kJ/g DM. Values for *Atriplex nummularia* leaves averaged 46.2 % and 4.95 kJ/g DM and for branches averaged 42.49% and 5.19 kJ/g DM, and for *Atriplex halimus* leaves averaged 6.30 kJ/g DM. Thus, differences among species were noted, with *Atriplex* having higher metabolizable energy yields than *Cassia*. This occurred even though the gross energy of *Cassia* was higher than that of *Atriplex*, indicating lower digestibility of *Cassia* than *Atriplex*. Ash content of *Atriplex* was higher than *Cassia* with little difference within species; crude protein content was higher in *Atriplex* than *Cassia*. The organic matter digestibility and metabolizable energy yield of *Atriplex* were similar to those of wheat straw; those of *Cassia* were somewhat lower.

Table 1. Composition and *in vitro* metabolizable energy of leaves (L) and small branches (B) of *Cassia sturtii* and *Atriplex nummularia* in Israel. Ash, crude protein (CP) and neutral detergent fiber (NDF) are in percent dry matter (DM). Organic matter digestibility (OMD) is in percent organic matter and gross energy (GE) and metabolizable energy (ME) yields are in kJ/g DM.

Plant	I.D.	Ash	CP	NDF	GE	OMD	ME
Cassia sturtii	1D 2.3 (L)	9.63	8.54	24.52	18.78	37.57	4.04
	1D 2.3 (B)		7.54			38.22	4.25
	1D 2.5 (L)		8.73	24.52		39.05	4.30
	1D 2.5 (B)		6.78			37.33	4.15
	1D 3.3 (L)	8.75	9.22	25.69	19.05	39.39	4.32
	1D 3.3 (B)		6.60	55.64		35.46	3.82
	4C 3.6 (L)		8.63	25.00		39.11	4.32
	4C 3.6 (B)		6.74			38.89	4.45
	8D 4.4 (L)	10.78	9.54	23.73	19.06	38.39	4.10
	8D 4.4 (B)		7.51			36.73	3.97
	8D 6.8 (L)	9.78	9.20	26.38	18.37	39.17	4.28
	8D 6.8 (B)		7.51			38.59	4.32

Continue Table 1.

Plant	I.D.	Ash	CP	NDF	GE	OMD	ME
Atriplex nummularia	3D 2.5 (L)					45.73	5.03
	3D 2.5 (B)		6.20			42.68	5.14
	5D 4.3 (L)	28.00	16.43	28.44	13.83	46.66	4.86
	5D 4.3 (B)	6.19	7.48	68.75	16.54	42.29	5.23
Atriplex halimus	(L)	34.22	16.10	27.32	11.09	48.57	6.30
Alfalfa		16.48	17.15	40.58	14.56	58.49	7.20
Wheat straw		11.89	5.05	61.85	14.88	45.35	5.82

Conclusions: In general, metabolizable energy yield is low for all shrubs; however, the shrubs could be an important source of crude protein and could be used as fodder when forage is scarce.

E.3 *In vitro* studies (Israel)

Various methods, treatments and tissue sources were applied to explore the morphogenic or embryogenic potentials of explants of *Atriplex nummularia, A. canescens, Cassia sturtii, H. aphyllum* and *Boscia foetida,* toward evolving systems for the efficient micropropagation and/or multiplication via somatic embryogenesis of each of these species.

E.3.1. Atriplex nummularia

Internode segment and leaf culture: 0.5-1.0 cm long internode segments and leaf discs, harvested from *in vitro* grown plantlets *of A. nummularia*, were cultured on MS medium containing 2,4–D (0.5, 1.0, 2.5, 5.0, 10.0 mg/l) and the cytokinins BA (1.0 mg/l) or Kin (1.0 mg/l), individually or in combination.

The internode segments responded by proliferating callus while the leaf discs produced only little callus from the cut ends and turned brown within 20 days. The greatest amount of callus developed from internode segments on MS medium containing 2.5 mg/l 2, 4-D. Increasing the concentration of 2,4-D produced spreading callus development on the entire surface of the explants. In the absence of 2,4-D only swelling was observed on the cut ends of the explants.

Incorporation of 1.0 mg/l BA in the 2,4-D-containing medium (0.5 or 1.0 mg/l 2,4-D) resulted in further swelling of the internode segments, which became glossy green on the surface. Callus did not develop, but after 4 weeks some elongated structures were observed, which failed to develop into any differentiated structures. The addition of 1.0 mg/l Kin to 2,4-D-containing medium elicited different responses than did BA. Light green calli developed from the entire surface of internode segments, with greatest callus development on medium containing 2.5 mg/l 2,4-D. In the greatest concentrations of 2,4-D (5 and 10 mg/l) plus 1.0 mg/l Kin the explants split horizontally and gave rise to large, light green, spherical calli.

Internode segments and leaf discs were cultured in the presence of NAA (0.1, 0.2, 0.5, 1.0, 2.0 mg/l) alone or in combination with 1.0 mg/l Kin. The leaf discs produced little callus in the presence of 1.0 and 2.0 mg/l NAA. Callus induction and root initiation occurred on internode segments in medium containing only NAA, while good callusing was observed in the presence of Kin. Callus induction from node segments was greatest in 2.0 mg/l NAA. The calli were white, glossy and globular. The presence of Kin in the medium prevented root initiation.

Callus from cultured internode and leaf explants: Calli derived from the above experiments were transferred to MS medium containing 1 mg/l of the auxins 2,4-D, NAA, IAA or IBA, along with 0.1 mg/l of the cytokinins BA, Kin or 2ip. Calli derived from internode segments and leaf discs responded similarly in terms of the quality and quantity of callus produced. Among the different auxins used, 2,4-D elicited the greatest amount of callus proliferation; the other auxins produced glossy calli along with root initiation. Up to 90% of the calli produced roots on medium containing NAA. Irrespective of the auxin used, calli on 2ip produced the greatest amounts of glossy, small callus. None of the treatments induced somatic embryogenesis.

Responses to TDZ of callus derived from internode segments: Callus generated from internode segments on medium containing 2,4-D plus kin or BA were transferred to various concentrations of TDZ (2, 5, 10, 20 and 40 μM). Increasing concentrations of TDZ helped to increase the rates of proliferation of small, glossy cells but did not induce somatic embryogenesis.

Effects of hormone-free medium, low auxin concentrations and TIBA on callus from internode segments: Callus generated on internode segments was transferred to hormone-free BRP medium or BRP medium containing 0.1 mg/l 2,4-D alone or in combination with 0.1 BA or 0.1 mg/l BA plus 1 mg/l TIBA. After 6 weeks on hormone-free medium, green, glossy, compact calli turned red. However, in the presence of 0.1 mg/l 2,4-D, calli were more glossy and light green in color. BA enhanced the glossy nature and reduced the pigmentation. On the combination of TIBA and BA, relatively large cells developed. None of the treatments, however, induced somatic embryo development.

Effects of increasing cytokinin with low auxin concentrations on callus from internode segments: Callus was transferred to MS medium containing 0.1 mg/l 2,4-D or NAA along with various concentrations of BA (0.25, 0.5 and 1.0 mg/l) or 0.1 mg/l 2,4-D along with a range of concentrations of TDZ (0.1, 0.5 and 1.0 μM). Calli in hormone-free medium were green and glossy, with minimal red pigmentation. Incorporation of 0.1 mg/l 2,4-D induced development of friable, light green callus with red pigmentation. Callus development in medium containing TDZ and 0.1 mg/l 2,4-D varied with the TDZ concentration. On 0.1 μM TDZ the calli were friable and highly pigmented while on higher TDZ concentrations they were green, compact and hard with minimal pigmentation. Callus on NAA-containing medium was light green, glossy and compact. The addition of 1 mg/l BA limited the development of pigmentation as compared to that in the lower concentrations used. Finally, no treatment induced somatic embryogenesis.

Effects of hormone-free medium, low auxin concentrations and TIBA on callus derived from leaves: Callus developed from leaf discs was inoculated onto BRP medium containing different combinations of auxins and cytokinins. No treatment was found to be helpful in induction of somatic embryogenesis. Callus growing on hormone-free medium turned brown within 6 weeks. Calli cultured on 2,4-D were light green. Calli turned dark green in the presence of BA, whereas they browned in Kin-containing media. Calli cultured on IBA or NAA without BA also browned, with minimal rates of multiplication. Some development of unorganized structures occurred on 0.1 mg/l BA and Kin, but organ formation failed to occur.

ABA and PEG effects on internode- and leaf-derived callus: The combination of ABA and PEG proved helpful in pine somatic embryogenesis (Klimaszewska et. al. (1997). ABA appears to play a role in maturation of somatic embryos while PEG increased the osmolarity of the medium. Therefore, callus generated on internode and leaf segments was transferred to medium containing these compounds.

Callus produced on internode sections was transferred to MS medium containing a range of concentrations of ABA (10,20,40 μM) alone or with 5% or 10% PEG. Callus growing on the control medium showed higher rates of multiplication and less pigmentation than that on any concentration of ABA used. Increasing concentrations of ABA increased the glossy nature of small cells. No significant difference was observed among the three concentrations of ABA used. The addition of 5 and 10% PEG caused desiccation of the callus but did not affect its proliferation. Callus multiplication occurred in all combinations of ABA and PEG; however, no differentiation occurred.

Callus produced on leaf discs was transferred to BRP medium containing 20 or 40 μM ABA alone or in combination with 5 and 10% PEG. After 3 weeks, calli on 20 μM ABA turned brown while 10% of the calli growing on 40μM ABA remained green. Callus transferred to 5 or 10% PEG also turned brown and died rapidly. Thus, it would appear that callus derived from leaves is less resistant to stress than that derived from internode segments.

Effects of hormones in liquid medium on callus derived from internode segments: Callus from internode segments was transferred to MS liquid medium containing 0.1 mg/l 2,4-D or NAA along with various concentrations of BA (0.25, 0.5 and 1.0 mg/l) or 0.1 mg/l 2,4-D along with various concentrations of TDZ (0.1, 0.5 and 1.0μM) and cultured on a orbital shaker at 80 rpm for 3 weeks. The calli in all treatments grew well. The clumps of cells that had settled on the base of the medium were filtered, washed with autoclaved doubled distilled water and plated on hormone-free semi-solid MS medium. Red pigmentation appeared after 3 weeks and by the 6th week most of the cells had turned red and died.

Seedling parts

Effects of auxins and cytokinins on seedling parts: Hypocotyls, cotyledons, leaves and roots, harvested from 2 week old in vitro grown seedlings, were cut into 0.5 cm lengths and cultured on MS medium containing 2,4-D, NAA or IBA (0.25. 0.5, 1.0, 2.0, 4.0 and 8.0 mg/l) or BA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/l) alone or in combination with 0.2 mg/l Kin. All explants cultured on hormone-free medium swelled at the cut ends but did not produce callus. The greatest amount of white globular fragile callus development on hypocotyl, root and cotyledon explants, which occurred from the entire surface, developed on 0.5 mg/l 2,4-D. NAA or IBA initiated roots from hypocotyls while new roots developed on root explants. BA and Kin combinations caused callusing on leaf discs only; the greatest amount of friable, white and elongated callus proliferation occurred on 2 mg/l BA plus 0.2 mg/l Kin.

After 5 weeks of culture callus derived from hypocotyls and cotyledons turned brown. Callus derived from roots, however, started developing compact, white, light green globular shaped structures, which showed morphological similarities early globular stage embryos. Therefore, the calli were transferred to hormone-free half- and full-strength MS and BRP media. Further development of the globular structures, however, was not achieved.

Effects of nitrogen source, cytokinins, osmotic stress: In the previous experiment, callus proliferation and globular callus development was greatest from roots, hypocotyls and cotyledons on MS medium containing 0.5 mg/l 2,4-D, but efforts to develop somatic embryos from the callus failed. Therefore, an alternative effort to induce somatic embryo development was made by incorporating several nitrogen sources, a high level of sucrose, ABA or various cytokinins into MS medium containing the same hormone concentrations.

Hypocotyls, cotyledons and root explants were harvested from 10-day old *in vitro* grown seedlings. Explants were placed horizontally on MS medium containing 0.5 mg/l 2,4-D along with one of several nitrogen sources at 5 mM concentration (glutamine, proline, alanin) or with 500 mg/l casine hydrolysate or with various cytokinins (0.1 mg/l BA or Kin or 40µM TDZ) or 40µM ABA or 60 mg/l sucrose. No significant difference was observed in callus quality following culture on the various nitrogen sources used. Addition of Kin increased callusing on roots, hypocotyls and cotyledons. TDZ also helped to increase callus production. ABA and a high concentration of sucrose did not impact significantly on callus quality. Finally, none of the treatments supported any morphogenic development from any explant type.

<u>Zygotic embryos:</u> Zygotic embryos of *A. nummularia* were extracted aseptically and cultured on variations of MS medium.

<u>Effects of 2,4-D and cytokinins on zygotic embryo explants:</u> Zygotic embryos were cultured on medium containing various concentrations of 2,4-D (0.5, 1.0, 2.5 and 5.0 mg/l), or containing 1 mg/l 2,4-D along with 0.1 mg/l BA or Kin or 2ip or in combinations of cytokinins (1mg/l BA plus 0.5 mg/l Kin or 0.1 mg/l BA plus + 0.1 mg/l Kin).

One day after inoculation, embryos developed cotyledons and hypocotyls in all treatments except the BA plus Kin-containing medium. Within one week the zygotic embryos developed into seedlings without roots. After 10 days these seedlings were subdivided and transferred to fresh medium of the same composition. 50% of test tubes were kept in darkness and 50% in light. Compact, shiny light green callus with elongated cells developed on all concentrations of 2,4-D. Callus proliferation was greatest on 1 and 2.5 mg/l 2,4-D. BA induced green callus containing large cells while Kin produced small brown cells. 2ip increased the glossiness of callus. Explants on 0.1 mg/l BA plus Kin developed large, translucent, white callus. No treatment caused somatic embryo induction. Elongated suspensor like cells developed on 2,4-D containing media, similar to those that occur during development of somatic embryos or pro embryos. The explants were kept in the same medium for 8 weeks but further development was not achieved. The efforts to develop these elongated cells into somatic embryos are continuing.

<u>Effects of hormone-free medium and low concentrations of auxin and cytokinin on callus derived from zygotic embryos:</u> Elongated suspensor-like callus transferred to hormone-free MS medium turned brown. The transfer of calli to 0.1 mg/l 2,4-D alone or in combination with 0.1 mg/l BA caused further callus proliferation. Root initiation from callus began when callus was transferred to MS medium containing 0.1 mg/l NAA plus 0.1 mg/l Kin. None of the treatments induced somatic embryogenesis.

<u>Effects of ABA, PEG and betaine on callus derived from zygotic embryos:</u> Attempts to generate somatic embryos failed when callus was transferred to MS medium containing ABA (40 & 80 µM) or 40 µABA plus 10% PEG or 1000 mg/l betaine. Callus in all treatments began with extensive root initiation and subsequently turned brown.

E.3.2. Atriplex canescens

<u>Micropropagation:</u> Stem sections were disinfested, cut into 2-3 cm long pieces each of which included 2-3 pairs of buds and inoculated onto MS medium containing 1 mg/l BA plus 0.1 mg/l IAA. Shoots that developed *in vitro* from buds initially appeared to be green and healthy, but turned brown after the first subculture. Unsuccessful efforts to alleviate the browning include sub-culture to fresh medium every 3 days and transfer to hormone-free MS medium or to the same MS formulation including hormones, plus one of the following: (1) 50mg/l ascorbic acid + 25mg/l citric acid + 25mg/l glutamine; (2) 100 mg/l each ascorbic and citric acids; (3) 20 µM AgNO3; (4) 1 mg/l activated charcoal

The shoots were cultured in each of these media for 3 weeks, but no improvement occurred in their growth or appearance. The problem was solved by the addition of salt to the medium. Shoots were transferred to hormone-free MS medium supplemented with 0.5% salts (0.25 % NaCl + 0.25% Na_2SO_4). The shoots turned green, which showed the affinity to salt of this halophyte species. Rooting of *A. canescens in vitro* had been accomplished by Mei, et al (1997) in MS medium containing 0.5 mg/l IBA and 0.1 mg/l GA_3. Therefore, shoots produced in the above treatment were transferred to the same salt-supplemented medium (MS + NaCl + Na_2SO_4) with that root-inducing formulation. Rooting began after 10 days and by day 18 50% of the plantlets had rooted.

<u>Callus proliferation from leaf discs</u>: Leaf discs (0.5 cm in diameter) were cultured on MS medium containing 1 mg/l of auxin (2,4-D, NAA, IBA, dicamba, or picloram). The leaf discs cultured on hormone-free medium turned black within 3 weeks. The greatest amount of compact, shiny, off-white colored callus developed from 100% of the explants on MS medium containing 1 mg/l 2,4-D. Explants on NAA and IBA turned black. 30-40% of the explants callused on explants cultured on MS plus 1 mg/l dicamba or 1 mg/l picloram.

E.3.3 Cassia sturtii

Mixed responses were initially found in terms of development of shoot primordia and embryo-like structures on calli developed through mature zygotic embryos. In these experiments, the zygotic embryos were first grown on MS+ 2.5 mg l^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D) medium followed by a 6 weeks incubation on MS + 40µM Thiadiazol (TDZ) medium.

Histological observations confirmed the initiation of somatic embryo development. Cells with embryogenic initials, globular structures and transition of globular to heart shape stages were seen on sections prepared from calli growing on MS medium containing 40µM TDZ.

Different effectors were applied to induce bipolar (with shoot and root) somatic embryos i.e manipulation of auxins and cytokinins combinations, nutrient and osmotic stress and application of amino acids. The effects of various treatments on callus derived through zygotic embryo explants is summarized in the following table.

No.	Experiments	Results
1.	Effects of 5 and 50µM of different cytokinins (TDZ, BA , Kin and 2iP)	50µM TDZ was found most effective in terms of maximum shoot proliferation along with scanty embryo development, TDZ was found to be more effective than BA
2.	Effects of different cytokinins (TDZ, BA , Kin and 2iP) along with different auxins (2,4-D, NAA and IAA)	Any treatment did not help in bipolar embryo development, although IAA was found to be helpful in maximum shoot development
3	Stress Effects of half strength MS along with BA or TDZ (5 and 40µM) Effects of elevated level of Sucrose (3,4,6 and 9%)	 Multiple elongated shoot development occurs along with very few embryos developing in medium containing TDZ Calli development in all treatments, but without BA and TDZ calli turned black and died while maximum shoot proliferation occurred on 3% sucrose along with 5µM TDZ, but did not help in bipolar embryo development
3.	Effects of different media (MS, BRP and WPM) along with 2,4-D	In the first stage of experiment (callus induction) WPM gave rise to maximum callusing compared to MS and |BRP; experiments are continuing with the second stage (incubation of callus on TDZ or BA enrich media) also.
4.	Effects of Amino acids (2.5 and 5 mM) Proline, L-Alanine, Glutamine and Casein hydrolysate along with 10µM BA or TDZ	Frequency of somatic embryo was significantly higher than amino acid lacking medium. Higher frequency of globular, elongated, cotyledonary stage embryos were seen on the callus. These structures were easily detached from the callus and in some cases, root initials were also seen. Highest frequency of overall proliferation (somatic embryos and shoot) was observed in medium containing proline.

Effects of different auxin on callus induction and further shoot morphogenesis : Experiments were undertaken to study the effects of different auxins at the first stage of callus development. Mature zygotic embryos were aseptically inoculated on MS medium containing 1 mg/l of Picloram, Dicamba or 2,4-D. Within 20 days of inoculation about similar frequency of callus development occurred on Picloram and Dicamba, however, the callus frequency was lower in 2,4-D. Calli were transferred onto MS medium containing different concentrations (5µM and 50µM) of TDZ and BA. After 4 weeks, calli derived from Picloram or 2,4-D showed only green unorganized structures on TDZ containing medium. These calli were again transferred onto half and full strength hormone-free MS medium containing 2, 3, 4 or 6% sucrose. Shoot proliferation from calli occurred on all treatments, however, highest shoot proliferation along with maximum shoot length were observed in half strength MS medium containing 2% sucrose. An unsynchronized pattern of somatic embryo development was achieved, but the embryos were developed only either with cotyledons or in some cases with shoot and root.

E.3.4 Haloxylon aphyllum

BRP medium, supplemented with 1.0 mg/l 2,4-D and 0.1 mg/l BA (medium A), was found to be the most suitable of the media formulations tested for induction of callus from hypocotyls and cotyledons of *H. aphyllum*. Globular and spherical, translucent, glossy structures, resembling embryogenic calli, were observed following the transfer of calli to hormone-free media. Efforts to induce somatic embryogenesis from these calli, including the application of auxins, cytokinins, TDZ, flurprimidol and ABA, as well as stress from microsalt and mechanical sources did not lead to somatic embryogenesis. Calli were generated from callus that had been subcultured repeatedly, as well as from seedling root, shoot and hypocotyl explants and immature zygotic embryos.

Calli generated from hypocotyls were subcultured at three-week intervals on medium A. It was then transferred to MS medium containing 0.1, 0.5 or 1.0 mg/l GA_3. After 4 weeks Globular structures developed on the upper surface of the callus, occurring at highest frequency in medium containing the greatest of these GA_3 concentrations, suggesting the possibility that this could lead to development of somatic embryos. Higher concentrations of GA_3 (1, 2.5 and 5 mg/l) were tested in BRP and MS medium. While callus did not show further growth on hormone-free media, increasing concentrations of GA_3 resulted in increasing rates of callus proliferation. Elongated, globular, translucent cells were generated on the callus but did not develop further, even after 3 months in the various GA_3-containing media.

Callus produced on medium A was transferred to semi-solid or liquid BRP medium containing low concentrations of various auxins along with cytokinins, as follows:

<table>
<tr><th colspan="4">auxin</th><th colspan="2">cytokinin</th></tr>
<tr><th>2,4-D, mg/l</th><th>IAA, mg/l</th><th>IBA, mg/l</th><th>NAA, mg/l</th><th>BA, mg/l</th><th>TDZ, mg/l</th></tr>
<tr><td colspan="4">-</td><td colspan="2">-</td></tr>
<tr><td colspan="4">-</td><td>0.5</td><td>-</td></tr>
<tr><td rowspan="6">0.25</td><td rowspan="2">0.25</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>-</td><td>-</td><td rowspan="3">0.5</td><td>-</td></tr>
<tr><td>-</td><td>0.1</td><td>-</td><td>-</td></tr>
<tr><td>-</td><td>-</td><td>0.1</td><td>-</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>-</td><td>1.0</td></tr>
<tr><td>-</td><td></td><td>-</td><td rowspan="4">0.5</td><td>-</td></tr>
<tr><td>-</td><td>0.25</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>-</td><td>-</td><td>0.25</td><td></td><td>-</td></tr>
<tr><td>-</td><td>-</td><td></td><td>0.25</td><td>-</td></tr>
</table>

Calli produced in the presence of 2,4-D was light green, glossy and friable, whereas callus growing on any of the other auxins but without 2,4-D was green and compact, and in the presence of 2,4-D and TDZ it was hard and dark green. BA helped to maintain the green color. None of the treatments induced somatic embryogenesis.

Elongated suspensor-like cells developed on medium containing IBA plus BA, suggesting a proembryonic process. Callus that developed in all treatments was transferred to hormone-free BRP, MS and LS media and kept under four different environmental conditions; in the culture room at 25 ± 2^{o}C under light, in the same environment but in darkness, at 4 ^{o}C under light and at 4 ^{o}C in darkness. Observations were recorded after 3 and 6 weeks. The callus produced on LS and MS media appeared the same but the rate of multiplication was greater on LS. On MS and LS media green, friable callus with large cells developed. Callus on BRP medium was more glossy, and the cells the smaller than those that developed on LS and MS. Compact, shiny, green callus with elongated translucent cells developed at high rates of multiplication on MS medium when the source of the callus was medium containing 0.25 mg/l 2,4-D plus 5μM TDZ. After 3 and 6 weeks of culture, no treatment found to lead to somatic embryo induction.

Efforts to culture *H. aphyllum* on liquid media were unsuccessful. Calli produced on semi-solid MS plus 2,4-D were transferred to liquid MS medium containing 1.0 mg/l 2,4-D or 1.0 mg/l NAA along with 0.1 mg/l BA, 0.1 mg/l Kin or 0.1 mg/l 2-iP. Within seven days the callus in all treatments turned brown. Similarly, calli raised on semi-solid BRP plus 1 mg/l 2,4-D and 0.1mg/l BA were transferred to liquid BRP medium supplemented with various auxin and cytokinin combinations. Seven days after inoculation, calli on hormone-free medium or on various auxins (IAA//IBA/NAA) plus 0.5 mg/l BA or only on 0.5 BA, turned brown. In 2,4-D containing medium, on the other hand, a few green clumps were observed, and on 2,4-D plus TDZ medium the calli were green.

<u>Salinity:</u> *H. aphyllum* is a halophyte. An experiment was conducted in order to determine the effects of salts on callus development, in which a range of concentrations (0.1, 0.5, 1.0, 1.5 and 2%) of sodium sulphate, sodium chloride and mixtures of the two in a 1:1 ratio with the same final total salt concentrations (0.1, 0.5, 1.0, 1.5 and 2%) were added to hormone-free BRP medium. Results were recorded after 3 and 6 weeks. In the control treatment, 50% of the cultures were green with good callus proliferation. As the salt concentrations increased, calli became more friable, less glossy and more dry. Calli cultured on increasing concentrations of NaCl became brown after 3 weeks. Among the NaCl treatments, The optimum concentrations of NaCl and Na2SO4 presented invdividually, was 0.1%, with callus proliferation at moderate rates. In NaCl, the callus was green in 30% of the cultures while in Na2SO4 80% of the cultures were green. When the two salts were mixed cultures remained green until the 6th week. The best results were observed with 1% combined salts, in which the highest rate proliferation occurred and 80% of the cultures were green.

<u>TIBA:</u> Exogenous auxin is required for somatic embryo induction but inhibits their further development. TIBA (2,3,5-triiodobenzoic acid) functions as an anti-auxin by interfering with polar auxin transport and block the availability of endogenous auxin. *Beta vulgaris* callus induced on auxin-containing medium and transferred to a TIBA- and cytokinin-containing medium induced somatic embryogenesis (Tatu et al, 1987, Kulshreshtha and Coults, 1997). TIBA also promoted somatic embryogenesis in tea. (Jacq and Sandsan, 1992).

Table 1: Effects of different salt concentrations on *Holoxyon aphyllum* callus growth after 6 weeks of incubation.

NaCl mM	**Na_2S_4 mM**	**% of salinity**	**% of green callus**	**degree of callusing**
-	-	-	50	+++
17.24		0.1	30	++
86.20		0.5	20	+
172.41		1.0	10	+
258.62		1.5	0	-
344.82		2.0	0	-
	7.04	0.1	80	++
	35.21	0.5	50	++
	70.42	1.0	30	+
	105.63	1.5	0	-
	140.84	2.0	0	-
8.62	3.52	0.1	90	++
43.10	17.60	0.5	77	++
86.20	35.21	1.0	80	++++
129.31	52.81	1.5	30	+
174.41	70.42	2.0	0	-

+ minimal callus; ++ little callus; +++ moderate callus; ++++ high callus

Callus raised on medium A was transferred to BRP medium containing various concentrations of TIBA (0.5, 1.0, 2.5 and 5.0 mg/l,) alone or 0.5 and 1.0 mg/l TIBA along with 0.5 and 1.0 mg/l of BA or Kin or 1mg/l GA_3. Callus cultured on hormone-free medium was green until the end of the 6th week. In the presence of lower TIBA concentrations calli were globular and elongated in shape and shiny and translucent, but in 5.0 mg/l TIBA all callus turned brown within 6 weeks. Callus on BA plus TIBA was green with large cells. That on the combination of TIBA plus Kinetin or TIBA plus GA_3 turned brown after 4 weeks. None of these treatments induced somatic embryogenesis.

<u>Polyamines:</u> Callus was transferred to BRP medium containing 20, 100 and 500 µM putrescine or to a control. Callus on the control medium and in the presence of 20µM putrescine turned brown after 6 weeks. Increasing concentrations of putrescine helped to maintain green shiny, glossy callus with some elongated cells. Callus cultured in 500µM putrescine proliferated into dark green, friable calli with the greatest rate of multiplication and remained green even 8 weeks after inoculation.

Although putrescine at the concentrations tested did not induce somatic embryogenesis, it did help to keep callus green and healthy for a long time. Therefore, other polyamines were tested. An experiment was conducted with three concentrations (10, 100 and 1000µM) of three polyamines (putrescine, spermidine and spermine) on medium A, which served as the control treatment for this experiment. Calli cultured on 10µM putrescine proliferated at a lower rate than on 10 µM of spermidine and spermine, but at 100 and 1000 µM there were no differences. Spermidine and spermine also caused development of much glossy callus with small cells.

<u>*In vitro* responses of seedling parts:</u> The potential of carrot to produce somatic embryos decreases with age (Robert et, al. 1997). Since the callus used in the above studies was initiated a year ago from hypocotyl explants and underwent repeated multiplication cycles prior to their use in these trials, we tested other tissue sources. The responses of hypocotyl and cotyledon explants on MS and BRP medium were recorded in previous work in this laboratory (Ramirez and Birnbaum, 2000). Hypocotyl and cotyledon explants were again used, in trials in which hormones that were not tested previously were applied. Root and internode segments were also used.

<u>2,4-D, BA and Kin effects on internode segments:</u> Internode segments were harvested from *in vitro*-grown seedlings 20 days after germination, cut into 0.5 cm lengths, placed horizontally on MS medium containing different concentrations of 2,4-D (1, 2.5 or 5.0 mg/l) alone or in combination with 0.1 mg/l BA or Kin.

Explants cultured on hormone-free medium showed only swelling at the cut ends. The greatest and most rapid green, compact callus production with some suspensor-like elongated translucent cells was observed on 2.5 mg/l 2,4-D. The addition of BA or Kin helped to keep the callus green. 4 weeks after inoculation, callus on 1 mg/l 2,4-D was green while that on 2.5 mg/l started to turn brown. The highest concentration of 2,4-D (5mg/l) did not help to increase callus production and the cells turned brown rapidly (Table 2).

Table 2: Callus induction on *H. aphyllum* internode segments cultured on MS medium; influences of 2,4-D, BA and Kin. Data were recorded 4 weeks after inoculation.

2,4-D (mg/l)	**BA (mg/l)**	**Kin (mg/l)**	**% of explants producing callus**	**Relative degree of Callusing**
-	-	-	-	+
1.0	-	-	100	+++
2.5	-	-	100	++++
5.0	-	-	90	+
1.0	0.1	-	100	++
2.5	0.1	-	100	++++
5.0	0.1	-	100	+++
1.0	-	0.1	100	+++
2.5	-	0.1	100	++++
5.0	-	0.1	100	+++

+ minimal callus ++ little callus, +++ moderate callus, ++++ high callus

<u>TDZ effects on hypocotyls, internode and root segments</u>: Hypocotyl, root and internode segments (0.5 cm long) were cultured on MS medium containing 5, 10, 15 and 20µM TDZ. Callus production was greatest from hypocotyl and root explants on 5µM TDZ, while from nodal explants it was greatest on 10 µM TDZ. The callus initiated from hypocotyls and roots was shiny, friable and dark green while that from nodal segments was compact, hard and dark green. 60 and 80% of the nodal explants browned on TDZ at 15 and 20µM respectively, while no browning was observed on the control medium and in 5 and 10µM TDZ. Although, the frequency of callusing from root explants was highest on 5µM TDZ, 15 and 20 µM promoted callus development on the entire surface of the explant (Table 3). TDZ did not promote any developmental process except for callusing.

Table 3: TDZ effects on *H. aphyllum* hypocotyl, internode and root segments cultured on MS medium. The data were recorded 4 weeks after inoculation.

TDZ (mM/l)	**Explant**	**% of explants producing callus**	**Relative degree of Callusing**	**Remarks**
-	hypocotyl	-	-	-
	Nodal section	100	+	Minimal callusing, only on cut ends
	Root	-	-	-
5	hypocotyl	100	++++	Shiny, dark green, friable callus, cells on upper surface brown and more glossy
	Nodal section	100	+	Dark green, glossy, compact
	Root	100	++++	Only from one cut end of the root, dark green, friable, glossy callus
10	hypocotyl	100	++	Callus same as previous treatment
	Nodal section	100	++++	Dark green, glossy, compact cells,
	Root		++	From cut ends of the root, dark green, friable, glossy callus
15	hypocotyl	100	+++	Callus same as previous treatment
	Nodal section	100	++	Dark green, glossy, compact cells, 60% explants shows browning
	Root		++	Callusing from the entire surface of explant
20	hypocotyl	100	+	Callus quality same as previous treatment
	Nodal section	100	++	Dark green, glossy, compact cells 80% explant shows browning
	Root		++	Callusing from the entire surface

+ minimal callus; ++ little callus; +++ moderate callus; ++++ high callus

Hormone-free media effects on callus from seedling explants: Callus generated from hypocotyls, internode segments and root explants was transferred to hormone-free SH, BRP, MS, White's and WPM medium. After 2 weeks in all media, callus derived from hypocotyls began to brown, while that derived from internode segments remained green. Callus generated from roots began with root development in all media except BRP. After 6 weeks on MS and White's medium all callus turned brown, while on SH and BRP shoot-derived callus was green and hypocotyl-derived callus was white. Callus on WPM showed the highest rate of multiplication. None of the media supported somatic embryogenesis.

ABA effects on seedling-derived callus: Callus from roots, hypocotyls and shoot segments was cultured on MS medium containing ABA (2.5, 5.0 and 10 mg/l) alone or in combination with 0.1 mg/l Kin. Callus on the control medium remained green until the 6th week. Increasing concentrations of ABA caused browning of hypocotyl and shoot-derived callus and the addition of Kin helped to maintain green color. Roots developed on 10% of the hypocotyl-derived calli in the presence of 10 mg/l ABA. Root derived callus turned brown and showed only root initiation.

Immature zygotic embryos

Since none of the attempts to induce somatic embryogenesis from hypocotyl, cotyledon and shoot explants elicited a positive response, and considering that for many species the embryo is the widely accepted explant for somatic embryo induction, mature zygotic embryos were tested as an explant source.

Hormone and media effects on callus induction and proliferation: Seeds were surface sterilized and seed coats removed aseptically under a stereo microscope. The intact embryos were cultured on half- or full-strength MS and on full-strength BRP hormone-free media or on these media containing 1.0 mg/l 2,4-D alone or in combination with 0.1 or 1.0 mg/l BA. Hypocotyl and cotyledon development began during the first week after inoculation. Embryos inoculated on hormone-free media produce no or little callus. Green, compact, friable callus developed from the entire embryo surface on MS and BRP medium containing 2,4-D alone or along with BA (0.1 mg/l or 1 mg/l). The presence of 0.1 mg/l BA increased the rates of callus multiplication and 1 mg/l BA helped to further increase development of dark green colored callus. The most compact and hardest callus developed on MS and BRP medium containing 1 mg/l 2,4-D with 1.0 mg/l BA.

After 3 weeks all the cultures were transferred to fresh medium containing the same hormone composition. After another 3 weeks the callus on MS or BRP medium containing 1 mg/l 2,4-D and 0.1 or 1.0 mg/l BA almost doubled in volume. During the six weeks of culture no significant differences were observed in characteristics of the callus that developed on BRP and MS media. Induction of somatic embryogenesis did not occur during the six weeks observation period.

Calli generated in the previous experiment, on BRP and MS media, were transferred to hormone-free BRP or MS medium; callus from each source was transferred to both of the media. Callus transferred to MS from either source turned brown much earlier than that transferred to BRP, but eventually all callus browned.

Effects of macro-salt stress on callus derived from zygotic embryos: Callus was transferred to hormone-free MS and BRP media, each with half of the normal concentration of macronutrients, in order to induce stress. After 4 weeks, translucent elongated structures developed on the callus growing on MS medium. After 8 weeks, roots had begun to develop on 35 % of the callus but further development of the suspensor-like cells was not observed. The same results were observed with BRP medium but the callus remained green for a longer period than on MS medium. After 12 weeks callus in all treatments turned brown.

Effects of NAA with BA on callus derived from zygotic embryos: Callus generated directly from zygotic embryos was transferred to MS and BRP media containing NAA 0.1 mg/l plus 1 mg/l BA. The callus on both media turned brown within 3 weeks of transfer. NAA proved not to be helpful even for callus maintenance.

E.3.2.5 Boscia foetida

Boscia species, trees native to Southern Africa, grow well under low rainfall conditions and provide good fodder. Rates of natural regeneration are low.

Tissue culture studies

Micropropagation: adventitious shoot regeneration from hypocotyls: Hypocotyl explants of *Boscia foetida* were cultured on 2 mg/l 2,4-D or IBA along with 0.2 mg/l l BA or on 2mg/l NAA plus 0.2 mg/l Kin on MS, BRP and B5 media. The highest frequency of adventitious shoot formation (18 shoots/explant) occurred on BRP medium containing 2mg/l IBA plus 0.2 mg/l BA. Regeneration had begun from both the distal and proximal ends of the explants as well as, in some cases, from the entire surface. Shoot initiation with proliferation of callus or roots from hypocotyl explants occurred on MS or B5 media with the same combination of hormones (2mg/l IBA +0.2 mg/l BA) at a lower frequency than from explants cultured on BRP medium.

Development of globular structures and further elongation from the cut surface of root explants or from the outer epidermis of the cotyledons indicates possibilities of either shoot regeneration or somatic embryogenesis. The development of globular, elongated structures and a tendency of detachment during subculture showed similarities with the known process of somatic embryo development.

Somatic embryogenesis: Development of globular structures on cotyledons: Cotyledon and root explants were cultured on MS, BRP and B5 media containing 2 mg/l 2,4-D or IBA along with 0.2 mg/l BA or on 2 mg/l NAA with 0.2 mg/l Kin. All explants cultured on the control medium turned brown. Globular and elongated structures developed on MS medium after 4 weeks, from either the cut surface or from the entire surface of cultured cotyledons. This response was observed in all the treatments on MS medium except the control. However, the effect of 2,4-D was found to be the most significant in terms of highest frequency of structure development from the entire surface of the explants (MS+ 4 mg/l 2,4-D+0.2BA). (Table 4).

Callus developed from cotyledon explants cultured on BRP and B5 media with 2,4-D, while callus and roots were generated in NAA and IBA. Root explants cultured on MS or B5 media containing any of the auxins tested (NAA, 2,4-D or IBA) developed globular structures from the cut surface of the explants.

Efforts to further develop the globular or elongated structures failed when the explants were transferred to fresh medium of the same composition or with lower concentrations of auxins (0.05 & 0.1 mg/l NAA, IBA or 2,4-D) along with 0.25, 1.0 or 2.5 mg/l BA. NAA and IBA only promoted more callusing while 2,4-D helped in maintaining the green globular structures. In another treatment when 2,4-D along with 1 µM TDZ was tested, an increase in the size of globular structures occurred but there was no further development.

Table: 4 Morphogenic responses of cotyledon, (C), hypocotyl(H) and root (R) explants of *Boscia foetida* after 4 weeks of culture.

Media	**MS**			**BRP**			**B5**		
Treatment / *Parts*	***C***	***H***	***R***	***C***	***H***	***R***	***C***	***H***	***R***
2 mg/l NAA	GR	GEE	E	C, R	R, C	-	C	C	G
4 mg/l NAA	G	-	GR	C	C	C	G, E	C	G
2 mg/l 2,4-D	G	G	G	C	C	C	-	C	G
4 mg/l 2,4-D	G*	GE	G	C	C	C	C	-	G, R
2 mg/l IBA	C,G	C,G,ES	-	C,R	S, R	R	C	C, G, E, R, S	G
4 mg/l IBA	C,G,R	C,G	-	C,R	-	R	C	G, E	-

C - callusing, G-Globular structures, E- elongated structures, S- shoot development, R-root development

* GS from entire surface of the explant

E.4 Propagation from seed (South Africa and Israel)

Considerable progress was made in improving rates of germination through a variety of seed treatments, including the influence of rumen digestion, on the production of seedlings of *Tetragonia* and *Salsolo*. Additional information has been accumulated on *Atriplex*, *Sutherlandia*, *Tripteris* and *Cassia* germination. Germination of *Cassia* and *Boscia* was improved by acid scarification treatment.

E.4.1 Cassia sturtii

C. sturtii seed germination was slow and inconsistent, beginning 20 days after inoculation and continued at a very low rate for a prolonged period. High rates of germination concentrated in a short time frame are necessary when uniformity in seedling size and physiological age are important for conventional nursery-based preparation of seedlings, and critical when seedlings are used to initiate tissue culture studies. Immersion in hot water, scarification by sulfuric acid and mechanical scarification were tested. Germination of non-treated seeds began 3 days after imbibition and was spread over an extended period, with only 18% germinating by day 10. Immersion in boiling water for one minute resulted in total loss of viability. The best results were attained by acid scarification; immersion in concentrated sulfuric acid for 30 minutes resulted in 75% germination within the first 4 days. Abrasion with coarse sandpaper was also effective, resulting in the germination of approximately 62.5% of seeds within 7 days.

E.4.2. Boscia foetida

Seed germination of *B. foetida* was also poor; low rates over a prolonged period. Improvement of rates of germination by mechanical and acid treatment was, therefore, undertaken. Seeds were mechanically abraded on no. 2 sandpaper or treated with concentrated sulphuric acid for 10, 20 and 30 minutes. Untreated seeds did not germinate until 20 days after inoculation, while about 10% of the seeds that were abraded mechanically germinated after approximately 15 days of incubation. Treatment with concentrated sulphuric acid for 30 minutes resulted in 80% germination within 7 days.

F) Impact, Relevance, and Technology Transfer

Graduate students and postdoctoral fellows worked on all aspects of the program at both Universities, with input of the BIDR collaborators in the work of the UP students. This program was extremely productive in terms of capacity building for technical staff and especially in terms of providing qualified post-graduates who will be in positions to make positive contributions to agriculture in South Africa. Several of the people who conducted their graduate research in this framework have already found employment.

It is felt that it is appropriate to recommend that farmers clear and fence areas in the dune valleys in the Kalahari and to plant *A. halimus*, *A. nummularia* and, perhaps, *C. strurtii*, for use as a fodderbank / drought reserve. We anticipate that larger areas of the various fodder species and selections will be planted and will be accessible to farmers. The frequent communication between the collaborating scientists has been valuable, as has input of the collaborators to the training of UP and BGU graduate students and technical staff. The program continues to contribute to the growing collaboration and good relations with the San or Koisan Bushmen and Afrikaner ranchers involved in animal husbandry and fodder shrub trials in the Northern Cape, who will benefit earliest and most immediately from the results of these studies.

G) Project Activities/Outputs

G.1.) Meetings

Havenga, C. and Rethman, N.F.G. 2003. The adaptation and distribution of *Boscia* spp. In the arid and semi-arid zones of Southern African. Proc. VIIth Int. Rangeland Congr., Durban, South Africa. 26 July – 1 August 2003.

Malan, P.J. and Rethman, N.F.G. 2003. Selection preference of sheep grazing different *Atriplex spp.* Proc. VIIth Int. Rangeland Congr., Durban, South Africa. 26 July – 1 August 2003.

Malan, P.J. and Rethman, N.F.G. 2003. Dry matter production and structure of four *Atriplex spp*. At three stages of maturity. Proc. VIIth Int. Rangeland Congr., Durban, South Africa. 26 July – 1 August 2003.

Rethman, N.F.G., Birnbaum, E., Degen, A. and Van Niekerk, W.A. 2003. The use of fodder shrubs to improve range conditions in arid areas. Proc. VIIth Int. Rangeland Congr., Durban, South Africa. 26 July – 1 August 2003.

Sharma, N.K. and Birnbaum, E.H. 2003. Breaking Seed Dormancy of *Cassia sturtii* R.Br. Desert Technology - 7, Jodhpur, Rajasthan, India, November 9 - 14, 2003.

Wilcock, T.E., N.F.G. Rethman and van Niekerk, W.A. 2004. The use of three fodder species for revegetation of acid zones. Poster at the 5th Int. World Agriforestry Congress. Orlando, U.S.A.

G.2.) Training

A UP graduate student, Christa Havenga, and technician, Annemarie Liebenberg, underwent training in tissue culture at the BIDR laboratory during this period, and transferred techniques and tissue cultures to UP. They have continued this work at UP, implementing the new methods in tissue culture studies. UP personnel, Ms E Ferreira, and graduate student, Annelize van der Baan, studied the gastest method for determination of digestibility of forages at BGU.

One postdoctoral fellow, three Ph.D., nine M.Sc. and two undergraduate students were trained as part of the program. One of the Ph.D. and all of the M.Sc. and undergraduate students are at UP. Descriptions of their work follow.

- <u>Francois Sparks</u> (M.Sc. Agric. Animal Science) studied the quality of edible material of *Atriplex* species and *Cassia sturtii*, including mineral composition and oxalic acid concentrations.
- <u>Linde Du Toit</u> (M.Sc. Agric. Animal Science) examined the energy supplementation of oldman saltbush, *A. nummularia*. He is now employed as a lecturer at the Pretoria Technikon.
- <u>Annelize van der Baan</u> (M.Sc. Agric. Animal Science) worked on analysis of the nutritive value of drought tolerant species.
- <u>Pieter Vermaak</u> (M.Sc. Agric. Animal Science) addressed the degradability and fermentation of fodder shrubs.
- <u>Danie Venter</u> (M.Sc. Agric. Pasture Science) studied the productivity and relative palatability of a range of fodder species, concentrating on field evaluations in the Northern Cape. His involvement in the program has helped to obtain a position in mineland reclamation.
- <u>Trove Wilcock</u> (M.Sc. Agric. Pasture Science) studied the development of *Cassia* and *A. nummularia* seedlings and compared *Cassia* with indigenous species. She supervised germination studies under-graduate students, produced planting material and established a paddock of *Sutherlandia*, at Hatfield, using seed collected by the team during field visits.
- <u>Christa Havenga</u> (Ph.D. Pasture Science) evaluated such interventions as water-harvesting from bare areas, stone mulches to reduce evaporative losses, water absorbent polymers to improve water holding capacity and use of thorn branches to protect plants against herbivory, using two tree species, *Rhus* and *Ziziphus* planted on bare areas, in addition to her work on the germination and *in vitro* propagation of *Boscia*.
- <u>Leendert Snyman</u>, (MSc, Animal-Science) was already involved in this program while an undergraduate, when he conducted a comparison of the effect of different treatments on the germination of *Suterlandia micorphylla*. His masters work was a study of the factors which influence inter-plant variation in palatability of *Atriplex numularia*, and possible relationships with digestibility and selected ruminal parameters.
- <u>Abuberker Hassen</u> (PhD, Pasture-Science), a PhD student from Ethiopia, identified several shrub species of *Indigofera*, which have low or no anti-quality factor present and which are well adapted to drier ecological areas.
- <u>Julius Tjelele</u> (M.Sc. Animal-Science) studied the nutritive value of *Indigofera* accessions.
- <u>Jacqui Els</u> (MSc, Agric Animal-Science) harvested hay from *Cassia sturtii* and *Sutherlandia microphylla*, to compare with alfalfa hay in terms of a range of nutritive quality parameters.

- Hanlee van der Walt and VL Marcantuono (undergraduates) were involved in germination studies of indigenous species.
- Andrei Korchagin (M.Sc.) student from Kazakhstan at BIDR, worked on *Atriplex* and *Cassia* propagation by root cuttings and by micropropagation, completed his work and earned his degree.
- Lenka Langhansova, (Ph.D.) from the Czech Republic did postdoctoral work on micropropagation and embryo and callus culture and regeneration at the BIDR lab.
- Shaher El-Mecawi, (Ph.D.), a Bedouin student at BIDR, examined the energy yield of the plants using a gastest.
- Nagendra Sharma, a postdoctoral fellow at BIDR worked on micropropagation, callus culture and regeneration, and somatic embryogenesis of several of the woody target species.

G.3.) Publications

du Toit, C.J.L., W.A. van Niekerk, N.F.G. Rethmanand R.J. Coertze (2004). The effect of type and level of carbohydrate supplementation on intake and digestibility of *Atriplex nummularia* cv. De Kock. S. Afr. J. Anim. Sci, 34 (Suppl 1):35-37.

Havenga, C.J., W.A. van Niekerk, N.F.G. Rethman and R.J. Coertze (2004). Certain qualitative characteristics of *Boscia foetida* at different sites in South Africa. S. Afr. J. Anim. Sci, 34 (Supplement 1):62-64.

van Niekerk, W.A., A. van der Baan, N.F.G Rethman, R.J.Coertze. (2003). The determination of digestibility of *Atriplex nummularia* cv. de kock (Oldman's Saltbush) by using two different inocula in an *in vitro* analysis. Tropical and Subtropical Agroecosystems. 3:337-339

van Niekerk, W.A., C.J.L. du Toit, N.F.G. Rethman, R.J. Coertze. (2003). The digestibility of *Atriplex nummularia* cv. de Kock (Oldman's Saltbush) fed to sheep if supplemented with different energy sources. Tropical and Subtropical Agroecosystems. 3:341-342.

van Niekerk, W.A., P.J. Vermaak, N.F.G. Rethman1 and R.J. Coertze (2004). Comparison of chemical composition of *Atriplex* spp. grown under South African conditions with regard to site, species and plant parts. S. Afr. J. Anim. Sci., 34(Supplement 1):98-100.

van Niekerk, W.A., C.F. Sparks, N.F.G. Rethman and R.J. Coertze (2004). Qualitative characteristics of some *Atriplex* species and *Cassia sturtii* at two sites in South Africa. S. Afr. J. Anim. Sci, 34 (Supplement 1):108-110.

van Niekerk, W.A., C.F. Sparks, N.F.G. Rethman and R.J. Coertzev (2004). Interspecies and location variation in oxalic acid concentrations in certain *Atriplex* species and *Cassia sturtii*. S. Afr. J. Anim. Sci, 34 (Supplement 1):101-104.

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H) Project Productivity

This project accomplished the proposed goals. In addition, it contributed in two major areas:

Affirmative Action: Women, members of disadvantaged ethnic groups in each country and people of other national origins played significant roles in the project. A postdoctoral fellow at BIDR is an Indian national. One of the UP Ph.D. students is a woman; the other, a gentleman from Ethiopia. An Israeli Ph.D. student is a Bedouin. Three of the UP M.Sc. students are women. One of the Israeli and three of the South African technicians are women. One of the Israeli technicians is an Bedouin. Each is well qualified for his/her particular role in the program. We feel that this broad representation of women and disadvantaged ethnic groups is an important spin-off contribution of the program.

Interaction with Farming Communities: Productive collaboration has been established with leaders in the farming communities. Our main cooperators in the field were Mr. Kolie van Wyk at Mier, Mr. Gert Olivier at Hotazel, Mr. Albert van Niekerk at Kenhardt and Mr. Willhem van Niekerk at Pofadder. The cooperation of these individuals was essential in conducting field trials and will be of great value in bringing the results to these communities for implementation.

I) Future work

Atriplex nummularia, *A. canescens*, and *Cassia sturtii* proved to have potential as fodder crops in revegetation projects. Large field trials under natural grazing conditions are needed to assess the affect of these crops on livestock production.

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